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CULTURES OF LEUKEMIC BLOOD LEUKOCYTES

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The number of existing theories of the formation of blood is sufficient evidence that the understanding of the origin and the fate of blood cells is still confused. During the past fifteen years several investigators have employed the method of tissue culture for studying the behavior, origin and development potencies of leukocytes in vitro. The results have been gratifying, since the cells could thus be observed while isolated from such complicating elements as blood vessel endothelium and connective tissue cells.

The following study of leukocytes in leukemic blood cultures was undertaken with the purpose of investigating several points of interest to both hematologist and clinician:

1. Do leukemic blood cells in culture behave differently from normal leukocytes?

2. Does the lymphoblast of acute lymphatic leukemia in culture differ in its behavior from the myeloblast of acute myeloid leukemia?

3. Do the lymphocytes of chronic lymphatic leukemia react like those of acute lymphatic leukemia?

4. What are the developmental potencies of the small lymphocyte?

5. Can the observations of Timofejewsky and Benewolenskaja (1929) be corroborated; i. e., that the myeloblast (hemocytoblast) of acute myeloid leukemia is capable of producing monocytes, giant cells, fibroblast-like cells, plasma cells and cells of the erythroblastic and granulocytic series?

6. Is a monocyte-like cell found as a transitional form in both lymphatic and myeloid leukemia, and what further light can be thrown on the origin of the monocyte?

7. Are cultures of leukocytes of the large cell ("blast") type of leukemia of aid in facilitating the clinical diagnosis?

8. Which of the theories of the formation of blood are substantiated by the observations on behavior of leukemic leukocytes in vitro?

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LITERATURE

Normal blood leukocytes have been cultured with modern tissue culture technic during the past fifteen years by a number of investigators, most of the observations being made on blood of experimental animals. In 1915, Awrorow and Timofejewsky reported the results of cultivation of the blood leukocytes of the normal rabbit, dog and guinea-pig, in which they showed that lymphocytes and monocytes are capable of differentiating into polyblast-like and fibroblast-like cells.

Carrel and Ebeling (1922) found that pure cultures of ameboid phagocytic elements (which they called large mononuclear leukocytes) developed in cultures of chick blood; they were uncertain of the origin of these cells. They noted that the lymphocytes spontaneously disappeared after the first week; they thought that it was possible that these cells were transformed into the larger forms.

M. A. Lewis (1926), who used chick, rabbit, frog and human blood cultures, also reported observation of the development of phagocytes and later fibroblast-like cells. Lewis did not derive these elements from lymphocytes, but concluded that they had developed from the monocytes of the blood.

A. Maximow (1925) noted that in cultures of blood leukocytes inoculated with tubercle bacilli, both lymphocytes and monocytes hypertrophied into macrophages and epithelioid cells.

In 1928, Maximow published a comprehensive study in which he reported observations on all the different types of leukocytes in the chick, guinea-pig, rat, rabbit and monkey in supravitally stained living cultures and fixed and stained cultures. He pointed out the following significant facts: 1. The granular leukocytes began to degenerate shortly after explantation and at the end of forty-eight hours had distintegrated; the lymphocytes and monocytes were transformed by change of individual cells in the course of a few hours into large ameboid, phagocytic, dye-storing elements, which were identical in origin, structure and function with most of the polyblasts or mononuclear exudate cells as found in inflamed tissue. Mitoses occurred in the hypertrophying lymphocytes and monocytes, but were rare. 2. In this transformation into polyblasts, the lymphocytes passed through a monocyte-like stage. 3. The polyblasts developed into fibroblast-like cells and later into typical fibroblasts. In his last work (1928, 1929) he reported the development of reticular and collagenous fibers in 25 day old cultures of blood leukocytes. He concluded that the phagocytic and fibroblastic potencies of the lymphocytes of the blood were conclusively demonstrated.

Awrorow and Timofejewsky (1914) were the first to culture human leukocytes in cases of myelogenous leukemia. They observed that an intense migration of the explanted cells into the medium occurred; the

mature granular elements died out after a few days; the "myeloblasts," which they did not distinguish from lymphocytes and large mononuclear cells, rapidly enlarged and developed into phagocytes, and later into spindle cells, fibroblast-like cells and giant cells. Some of the myeloblasts were thought to change into myelocytes.

Timofejewsky and Benewolenskaja (1926-1929) reported a detailed study of the leukocytes in three chronic lymphatic, one subacute lymphatic, one atypical lymphatic, and two myeloid leukemias. The cultures were studied in the living condition and in sections. They found, in cultures of normal blood and in that of lymphatic leukemia, lymphocytes developing into monocyte-like cells, macrophages and fibroblast-like cells. The most noteworthy results were seen in their cases of myeloid leukemia, in which the majority of the explanted cells were classified as hemocytoblasts, i. e., myeloblasts. They observed the development of specific granules in the myeloblasts, and also the transformation of myeloblasts into erythroblasts. The height of granulopoiesis was reached in twenty-four hours, and that of erythropoiesis, in forty-eight hours. Both myelocytes and erythroblasts degenerated in from three to five days. On the basis of their investigations these observers concluded that:

1. Lymphocytes of normal blood and lymphatic leukemia can develop into macrophages, plasma cells, epithelioid cells, multinuclear giant cells and fibroblast-like cells.

2. Hemocytoblasts of acute myeloid leukemia can develop in vitro into macrophages, plasma cells, epithelioid cells, multinuclear giant cells, myelocytes, erythroblasts and normoblasts.

3. The lymphocytes of normal blood and of lymphatic leukemia and the hemocytoblasts of myeloid leukemia pass through a monocyte-like stage in the course of their development into macrophages.

Hirschfeld (1927) cultured the leukocytes of leukemia in human plasma, using those of three chronic (small cell) lymphatic, two acute myeloblastic and two myeloid leukemias; he reported conditions after twenty-four, forty-eight and seventy-two hours (in one case observations were made at six hours). From these studies he concluded that the cells of myeloid and lymphoid leukemia develop into fibroblast-like cells and macrophages, but that these are derived from the myeloblasts in the myeloid cases, and from monocytes in the lymphoid cases. Since no mitotic figures were seen, and since the percentage of monocytes in these lymphatic leukemias was admittedly small, Bloom (1928) and Timofejewsky and Benewolenskaja (1929) did not believe that the macrophages in Hirschfeld's experiments could have developed only from monocytes.

Hirschfeld and Klee-Rawidowicz (1928) cultured the blood cells in a case of lymphatic leukemia in which the blood consisted almost exclu-

sively of lymphocytes with a few monocytes. The lymphocytes were ameboid and formed pseudopodia; the migrating monocytes became greatly enlarged and took on the appearance of reticular cells. They concluded that the monocytes seemed to have increased greatly both in number and size.

Veratti (1928) cultured the leukocytes in two cases of myeloid leukemia and noted the development of cells with the properties of histiocytes, large giant cells and elongated cells. He believed that these cells were developed in normal blood from monocytes and in leukemic blood from monocytes and hemohistioblasts.

Bloom (1927) cultured the lymph from the thoracic duct of rabbits, which contains few cells other than small and large lymphocytes. As the lymphocytes hypertrophied they went through a monocyte-like stage and then developed into polyblasts and fibroblast-like cells. Bergel (1930) obtained similar results in cultures of rabbit lymph.

Parker and Rhoads (1928) reported observations on incubated normal and leukemic leukocytes. They noted that most of the polymorphonuclear neutrophils disintegrated after twenty-four hours, although some lived for a longer time and developed refractile granules. The eosinophils survived longer than the neutrophils and showed no degenerative change. The lymphocytes lived as long as the eosinophils and retained their normal characteristics throughout life; no increase in size of these cells was noted, and they were not phagocytic. However, in the ascitic fluid of a case of chronic lymphatic leukemia, in which most of the cells were typical small lymphocytes and in which there were no monocytes, large phagocytes were observed after the fluid had been incubated for six days. The authors pointed out that this finding bears out the work of Bloom on cultures of lymph.

TECHNIC

From 10 to 12 cc. of blood was drawn aseptically from the median basilic vein into a syringe containing 1 cc. of heparin (1:2,500 in 0.85 per cent sodium chloride solution) to prevent coagulation, and transferred to a sterile centrifuge tube. As soon as possible, although in some instances there was an interval of one or one and one-half hours because of the distance of the hospital from the laboratory, the blood was centrifugated at high speed and the plasma pipetted off. The buffy coat was then coagulated by the addition of from 1 to 3 drops of rabbit embryonic extract and undiluted rabbit plasma. After it had been allowed to clot in the incubator, the buffy coat was removed with a glass spoon, placed in a hollow slide containing from 2 to 3 cc. of Tyrode solution, and cut into very small pieces with a pair of fine, sharp scissors. The cultures were made in large hanging-drop slides as follows:

A minim (0.06 cc.) of Tyrode solution was dropped on a large square (40 by 40 mm.) coverslip, and a small round (21 mm.) coverslip was placed over it. A drop of diluted rabbit plasma with Tyrode solution and distilled water in the ratio of 4:2:1 was placed on the round coverslip, a small bit of buffy coat added, and the

drop coagulated by the addition of an equal amount of rabbit embryonic tissue extract diluted with 6 parts of Tyrode solution. After the plasma had coagulated, the coverslip was inverted over the hollow slide, the chamber sealed with petrolatum and a layer of paraffin, and the slides placed in the incubator. This is the Maximow method of tissue culture. From thirty to forty slides were made in each case.

For control material the remainder of the buffy coat was fixed immediately and carried through the same processes of fixation and staining as the cultures.

The cultures on the slides were observed in the living condition, fixed at six hour intervals or less during the first twenty-four hours, and at twelve hour intervals or less after that time. As the most significant changes occurred during the first two days, a special attempt was made to study these early changes in detail. The cultures were fixed in Zenker's solution plus formaldehyde solution, embedded in celloidin, serially sectioned and stained with hematoxylin-eosin-azure II solution.

CASE REPORTS AND OBSERVATIONS IN LEUKOCYTIC CULTURES

For the sake of brevity the case histories and necropsy findings are not given in detail, although careful study was made of each case.

CASE 1.—*Acute Lymphatic Leukemia.*—V. H., a girl, aged 4, a patient of Dr. Joseph Brennemann at the Children's Memorial Hospital over a period of nine months, during this time had three exacerbations of fever, lymphocytosis followed by leukopenia, splenomegaly and moderate lymphadenopathy. The remission after the first attack lasted three months, and after the second, six weeks. The terminal picture was that of acute lymphatic leukemia with the classic syndrome of lymphadenopathy, great enlargement of the liver and spleen, hemorrhages from the mucous membranes, necrotic areas in the mouth and throat and a lymphocytosis of 33,000. Within the twenty-four hours before death, large amounts of urates were excreted in the urine, the liver and spleen decreased markedly in size, and the total white count dropped to 1,000.

At necropsy, a dense lymphocytic infiltration of the bone marrow was found, with a less marked infiltration of the liver and kidney. The spleen and lymph nodes were moderately enlarged, and the germinal centers were actively proliferative. It was felt by three pathologists who examined the sections that the process was of a leukemic nature, although the extent of infiltration was less than that which is usually seen in acute lymphatic leukemia.

Observations in Leukocyte Cultures: The blood cell counts on the day of culture (May 11, 1931) were as follows: red, 3,600,000, with hemoglobin 45 per cent (Dare); white, 28,450; differential (Wright's stain), 2 per cent band-shaped and 1 per cent segmented polymorphonuclear leukocytes, 94 per cent small to medium-sized lymphocytes, 2 per cent leukocytoid lymphocytes, 0.3 per cent eosinophils, 0.3 per cent basophils, 1 per cent erythroblasts, and 10 per cent fragmentary cells.

The nucleus of the lymphocytes was oval, with a slight indentation on one or occasionally on both sides. The chromatin pattern was coarse and the nuclear membrane heavy. In the larger lymphocytes one or two nucleoli were seen. The nucleus almost completely filled the light blue cytoplasm, which contained a varying number of azure granules; in a few cells the cytoplasm was finely vacuolar. The lymphocytes varied in size from that of an erythrocyte to half again as large, and because of the great number of intermediate sizes it was almost impossible to separate the small from the medium-sized lymphocytes.

Control sections made from the bits of buffy coat remaining after the cultures were explanted were made up of a dense collection of erythrocytes, among which leukocytes were liberally distributed. In some areas the leukocytes formed dense clusters, and in others they were scattered sparsely among the red cells. The great majority of the leukocytes were small lymphocytes with a diameter equal to that of an erythrocyte, although a few were slightly larger. They had an oval nucleus with one or two indentations of the heavy nuclear membrane, a coarse chromatin pattern and basophilic, nongranular cytoplasm. The absence of cells of the monocyte type was noteworthy. Very few polymorphonuclear neutrophils and eosinophils were scattered in the control material. A few ameboid lymphocytes and polymorphonuclear neutrophils were seen at the periphery.

The control culture, fixed immediately after explantation without being incubated and approximately two hours after the blood had been drawn for culture, was made up of the same types of cells, consisting chiefly of small lymphocytes unevenly distributed among erythrocytes. In the less densely crowded areas, the cells appeared larger, and more of the medium-sized lymphocytes were seen. At the periphery of the tissue a few ameboid lymphocytes were found, having an elongated, oval shape, and plump, deeply indented nuclei. A few monocytoid cells were seen in this region of the explant. There were relatively few granular cells, although a very few polymorphonuclear neutrophils and a larger number of eosinophils were seen. Most of the eosinophils had two or three lobes to their nuclei, but a rare eosinophilic myelocyte was present.

After two hours, a few lymphocytes had migrated from the periphery of the explant; those at the outer edge of the zone of migration were elongated, irregular cells, each with a relatively large amount of nongranular cytoplasm and an oval nucleus; those nearer the explant were round or oval; each had more cytoplasm than similar cells within the explant, and an oval, slightly indented nucleus. Within the explant there had been definite hypertrophy of some cells, although in the great majority there had been little change. The hypertrophied cells each had an increased amount of cytoplasm and a large plump nucleus containing one or more nucleoli and showing an irregular nuclear membrane. There were several monocytes with horseshoe-shaped nuclei. More polymorphonuclear neutrophils were seen in this culture than in either the control tissue or the culture. Eosinophils were frequent.

After five hours, there had been little migration of the cells from the periphery of the explant, but there were ameboid forms within the explant. There were many medium-sized lymphocytes and an occasional macrophage with an elongated, crescent-shaped nucleus and nongranular cytoplasm containing vacuoles and cellular débris. There were many polymorphonuclear cells, in some of which the nuclei were pyknotic and broken. A few eosinophils were present. No mitotic figures were seen.

After twelve hours, most of the polymorphonuclear cells and many of the lymphocytes had pyknotic nuclei and degeneration vacuoles in the cytoplasm. While there were many unchanged small lymphocytes, the majority had increased further in size. Frequent monocyte-like and elongated cells, as well as dumb-bell-shaped or ameboid lymphocytes were seen. An occasional large phagocytic, nongranular cell contained ingested débris. In the less crowded areas a few large lymphocytes were found. No mitosis was observed. Frequent eosinophilic leukocytes and myelocytes were scattered throughout the section.

After twenty-one hours, there was a wide zone of migration about the explant, made up of ameboid mononuclear and very few polymorphonuclear cells. The majority of the cells were rounded and elongated lymphocytes with bizarre

nuclear forms, i. e., dumb-bell-shaped, rounded, triangular or knobbed. A few of these cells had the typical nucleus of a polyblast.

Within the explant, among the mass of medium-sized lymphocytes and degenerating cells, many larger lymphocytes (which corresponded with the larger, medium-sized lymphocytes of the lymphatic tissue) were found, each with a large, round, vesicular nucleus, two or three prominent acidophil nucleoli, and a thin rim of nongranular cytoplasm. There were also many medium-sized lymphocytes—larger than the explanted small ones—which had rounded cell outlines, but the nuclei of which were deeply indented on all sides. Many monocyte-like cells in which the nucleus-plasma relationship was equal were present.

After twenty-eight hours, large numbers of dying cells were present in the cultures. Those cells that were living were all larger than the ones originally explanted. There were many very large lymphocytes, having large round nuclei and deeply basophilic cytoplasm, and also large numbers of phagocytic cells containing ingested débris with irregular or crescent-shaped nuclei. Frequent monocyte-like cells, as large as a medium-sized lymphocyte, and a few cells typical of the large mononuclear cells were seen. Eosinophils were rare. The medium-sized lymphocytes had the deeply lobulated nuclei described. No mitotic figures were seen.

After forty-five hours, there had been a striking hypertrophy of the mass of living cells into large lymphocytes, monocyte-like cells and hemocytoblasts. Islands of living cells in which there were no mitotic figures were scattered among the less cellular areas in which there were many degenerating cells. These islands were made up of the following cell types: (1) a few small lymphocytes, most of which had an irregularly indented nucleus; (2) somewhat larger lymphocytes having the same irregular nucleus and a thin band of basophilic cytoplasm; (3) cells of the same size as these larger (medium-sized) lymphocytes in which the nucleus was horseshoe-shaped or kidney-shaped, and in which there was a large amount of cytoplasm (these may be called monocytoid lymphocytes); (4) somewhat larger cells with a horseshoe-shaped nucleus and a large quantity of slightly basophilic cytoplasm, which were monocytes; (5) very large lymphocytes with a round nucleus, two or three nucleoli and a narrow rim of basophilic cytoplasm; a few of the latter were very large lymphocytes with a large, kidney-shaped nucleus containing heavy chromatin particles, two or three acidophil nucleoli and a distinct nuclear membrane surrounded by a basophilic cytoplasm in which an attraction sphere might be seen opposite the indentation of the nucleus (see plate). These cells were identical in appearance with the hemocytoblasts of Maximow, Jolly (1923) and others. Scattered through the culture were many large lymphocytes and monocyte-like cells. There were also many single hemocytoblasts standing alone among grouped dying cells. The number of monocytoid cells in the cultures was strikingly high. Frequent eosinophilic leukocytes, but no neutrophils, were present throughout the culture. Occasional phagocytic cells were seen.

After fifty-three hours, the same cell types as described in the forty-five hour cultures were to be found. In addition, there were many large monocyte-like cells in which the nucleus was vesicular, containing a large amount of nuclear sap and widely separated chromatin particles. The number of phagocytic cells had increased. There were a few cells with a crescent-shaped nucleus and a vacuolated cytoplasm in which granules that appeared to be eosinophilic were arranged at the periphery of the cytoplasm. These were probably macrophages with ingested eosinophilic granules. A few eosinophils with pyknotic nuclei were scattered throughout the culture.

After seventy-two hours, the cultures showed little change. Many islands of living cells were grouped among the cellular débris. The number of monocyte-like

cells had increased; they were larger than in the earlier cultures. Some of the largest ones contained ingested material and were now obvious macrophages. Although many hemocytoblasts were present, they were less frequent than in the forty-five hour cultures. There were many elongated, fibroblast-like cells among the monocyte-like cells, each with a pale, vesicular, oval nucleus, prominent acidophil nucleoli and a polyhedral cytoplasm. An occasional very large cell with two large, vesicular nuclei with heavy chromatin particles, acidophil nucleoli and a deeply basophilic cytoplasm was seen. Mitotic figures were absent.

In the four and five day cultures, most of the cells had degenerated, only a few phagocytic cells and monocyte-like cells remaining.

In summary of the observations in the cultures of the leukocytes in this case of atypical lymphatic leukemia, in which the total white cell count on the day of culture was 28,450, with 94 per cent smaller lymphocytes, it may be said:

1. The lymphocytes were actively ameboid shortly after explantation.

2. The lymphocytes hypertrophied as early as two hours after explanation.

3. Early in this process of hypertrophy, the cells assumed a monocyte-like appearance.

4. After twenty-eight hours, large numbers of large lymphocytes, monocyte-like cells and a few macrophages were present.

5. After forty-five hours, the living cells were medium-sized lymphocytes, large lymphocytes, monocyte-like cells, large monocytes and hemocytoblasts, and a few eosinophils.

6. In the older cultures, the monocyte-like cells appeared to become epithelioid and the number of phagocytic cells to increase.

7. No evidence of erythropoiesis or of granulopoiesis was found.

The outstanding feature of this case is, then, the development of numerous large lymphocytes (typical hemocytoblasts or basophilic stem cells of Maximow) from the explanted small and medium-sized lymphocytes. In the absence of mitotic figures and in the presence of large numbers of transitional forms between small lymphocytes and the hemocytoblasts, one can only conclude that the small lymphocyte is capable of developing into the large basophilic stem cell which is normally found in the lymphatic tissue, and in the bone marrow.

CASE 2.—*Acute Myeloblastic Leukemia.*—C. P., an Italian, aged 36, was admitted to Cook County Hospital, Nov. 3, 1930, with a history of illness of seven months' duration, the initial symptoms of which were pain in the sacro-iliac region and fatigue. He had received roentgen treatment over the spleen at the Presbyterian Hospital in June, 1930. The acute symptoms began one month before admission, when anorexia, malaise and nausea developed. During the hospital course the picture presented was that of acute leukemia with progressive anemia, leukocytosis with large numbers of blast forms and myelocytes, hemorrhages from the mucous surfaces, generalized lymphadenopathy and splenomegaly. He died on Nov. 26, 1930.

The blood cell counts by the attending intern were as shown in table 1.

The anatomic diagnosis made at necropsy by Dr. R. H. Jaffé was as follows: chronic myelogenous leukemia; petechial hemorrhages in the mucous membranes, lips, skin, epicardium and endocardium, pleura and pulmonary parenchyma; diphtheritic colitis; hyperplasia of the cervical, axillary, inguinal, epitrochlear, periaortic, peribiliary and pancreatic lymph nodes; severe anemia of the internal organs.

Observations in Leukocyte Cultures: At the time of withdrawal of blood for the cultures, the cell counts were as follows: red, 1,960,000; white, 48,900; differential, 84 per cent myeloblasts, 1 per cent metamyelocytes, 12 per cent polymorphonuclear neutrophils, 2 per cent basophils, 1 per cent lymphocytes and 1 normoblast per two hundred leukocytes.

As seen in the dry smear stained with May-Grünwald-Giemsa stain, the myeloblasts were round cells varying in size from that slightly larger than an erythrocyte to one twice as large. The large nucleus had an almost imperceptible nuclear membrane and a fine chromatin arrangement with one or two nucleoli, about which

TABLE 1.—*Blood Counts in Case 2: Acute Myeloblastic Leukemia*

Date	Red Cells	Hemoglobin, per Cent	White Cells	Differential, per Cent								
				Myeloblasts	Promyelocytes	Myelocytes	Metamyelocytes	Polymorphonuclear Neutrophils	Small Lymphocytes	Basophils	Eosinophils	Nucleated Red Cells
7/11/30	4,500,000	84	108,000	..	..	31	..	65	1	3	...	..
7/12/30	4,860,000	87	97,000	..	..	31.4	..	47.6	3.3	1.4	0.9	..
11/26/30	1,570,000	50	41,950	81	3	0	1	12	1	2	...	1

there was a heavy condensation of chromatin particles. The separation of chromatin and parachromatin was not distinct. The nucleus was surrounded by a halo of pale blue, finely vacuolated cytoplasm, which was denser at the periphery, so that the cell was sharply outlined by a deep blue rim.

In the control cultures fixed immediately after explantation (about two hours after removal of the blood from the body), most of the cells were lymphoid. The majority were slightly larger in diameter than an erythrocyte. The nucleus was round or oval, contained two or three large particles of chromatin joined by delicate strands, and was sharply demarcated from the encircling narrow rim of basophilic, nongranular cytoplasm by a distinct membrane. At the periphery of the culture were a few ameboid cells. A few polymorphonuclear cells in which the fine neutrophilic granules were readily visible in the cytoplasm were scattered throughout the culture. A rare normoblast was seen in these control sections.

At the end of six hours, active migration was present around the periphery of the explant. At the outer edge of the zone of migration there were many polymorphonuclear leukocytes, the cytoplasm of which was filled with fine, almost imperceptible, reddish-purple granules. The majority of the ameboid cells had the appearances of the exudate mononuclear cells or polyblasts, as described by Maximow (1902-1928); they were characterized by an eccentric, irregular and wrinkled nucleus and irregular, cloudy blue cytoplasm, which assumed a variety of shapes. While most of the polyblasts were rounded, a few were elongated and had long triangular pseudopodia.

Scattered among these polyblasts were ameboid cells that had a single-lobed nucleus gathered at one end of the cell and an irregular, definitely granular cytoplasm. These granules were very slightly larger than those in the polymorphonuclear cells and stained dull red. These cells were recognized ameboid myelocytes. A very rare eosinophilic myelocyte was seen. Within the explant there had been hypertrophy of most of the cells, although those in the more crowded areas remained unchanged. The enlargement had occurred in both the nucleus and the cytoplasm, although the latter was more abundant than in the original cells, and the nucleus had assumed an eccentric position.

After twelve and eighteen hours, the zone of migration was wider and contained a variety of cell forms. The largest number of cells were rounded and elongated polyblasts, polymorphonuclear neutrophils, ameboid neutrophilic myelocytes and an occasional dying cell with a pyknotic nucleus. A few of the ameboid polyblasts contained ingested débris. Scattered among the ameboid cells were numerous neutrophilic myelocytes; in these cells, the nucleus was round or slightly indented, and the cytoplasm contained very fine neutrophilic granules; in many the cytoplasm bulged to one side of the nucleus. Numerous rounded myeloblasts were also present near the edge of the explant. In the zone of migration the outstanding feature was the large number of transitional forms between myeloblasts and ameboid polyblasts. Within the explant at this stage there had been hypertrophy of the majority of the cells; the nuclei were larger and more vesicular, and contained from one to three acidophil nucleoli, and were frequently deeply lobulated; the cytocentrum was prominent in many. A few cells had a relatively large amount of cytoplasm and were morphologically similar to monocytes. A few myelocytes were distinguished. Most of the cells that were not closely packed had assumed a slightly irregular, frequently elongated shape and appeared to be ameboid. No polymorphonuclear neutrophils were found within the explant; apparently these elements had all migrated into the medium. The culture was sparsely dotted with pyknotic nuclei of dying cells. At the edge of the explant, an occasional typical normoblast was seen, and in a few of these the nucleus was in the process of being extruded.

After forty hours, very few polymorphonuclear leukocytes were seen in the zone of migration, and most of those present had pyknotic nuclei. The polyblasts had increased in number and size; there were now many spindle-shaped and polyhedral cells with long cytoplasmic processes. Close to the explant were rounded polyblasts and relatively few neutrophilic myelocytes.

Within the explant were active phagocytes about the size of an erythrocyte; their cytoplasm contained débris, and their nuclei were crowded to one side by the ingested particles. However, most of the cells here were nonphagocytic, had large, round or oval, vesicular nuclei, each occupying about two thirds of the cell, and basophilic nongranular cytoplasm. There were a few mitotic figures within the explant. Scattered throughout the explant were many cells characterized by a deeply pyknotic nucleus and an acidophilic rounded cytoplasm, which resembled normoblasts. However, there was a great variety in the size of their pyknotic nuclei as well as in the size of the definitely acidophilic cytoplasm, from a diameter equal to that of an erythrocyte to half that and less. Because of the absence of transitional forms between the lymphoid type of cell through the basophil erythroblast and normoblast stages, and because of the variety in size and the absence of a sufficient number of mitotic figures, it seems clear that these were degenerating leukocytes rather than normoblasts. One or two cells were seen that were typical of polychromatic erythroblasts with a heavy "checker-board" chromatin arrangement of the nucleus. However, since the normoblasts in the smear of the blood cultured constituted 0.5 per cent, it is thought that the small number found in the

culture could be attributed only to explanted normoblasts and not to those that had arisen heteroplastically from a lymphoid precursor in the culture.

After sixty hours, migration had continued at a slower rate. The cells in the zone of migration had increased in size, and many of them were fibroblast-like. The nuclei were large and pale, with dustlike particles of chromatin arranged in a fine network; the nuclear membrane was smooth in contrast to the lobulated and wrinkled membrane of the small polyblasts, and one, or occasionally two, large nucleoli were prominently acidophilic. There were many cells similar to these elongated polyblasts, with irregular or plump, oval nuclei and an ameboid cytoplasm, which was pale gray and very fine, and contained reddish-purple granules. These were thought to be ameboid myelocytes. A rare polymorphonuclear cell was present with a somewhat pyknotic nucleus. There were many dead cells with shrunken acidophilic cytoplasm, and pyknotic nuclei were scattered among the polyblasts.

Within the explant were frequent mitotic figures. At the periphery the cells were slightly elongated, while in the center they had maintained their rounded shape. The nuclei were large and vesicular, contained one or two nucleoli, had a fine chromatin arrangement and a heavy, frequently deeply indented nuclear membrane. In many cells the cytoplasm was vacuolated and contained ingested particles. Sparsely distributed through the culture were deeply pyknotic nuclei of dead cells; again, in the areas where there were dense groups of erythrocytes these cells resembled normoblasts. But here, too, the absence of transitional forms from lymphoid cells and basophilic erythroblasts and normoblasts, and the variation in size of the cells, were evidence against erythropoiesis in vitro.

After eighty-eight hours, the elongated cells in the zone of migration had developed very long, slender, tapering processes. The cells were not as large or as long as in the sixty hour cultures, and the percentage of rounded and phagocytic cells was higher. Within the explant were many dead cells. Practically all of the living cells were macrophages, many of which contained phagocytosed débris. There were occasional binucleated cells, and many cells had a very large, deeply lobulated nucleus surrounded by a wide margin of vacuolated cytoplasm. No cells with specific granules were seen.

After four days, active phagocytosis was progressing, and as the leukocytes died and were ingested, clear areas remained, into which the polyblasts migrated, becoming then elongated and fibroblast-like. Most of the cells within the explant were very large with vesicular nuclei and vacuolated cytoplasm. Occasional mitotic figures were present.

After six and eight days, the cultures showed large numbers of dead cells, and in contrast with the closely packed cellular explant of the original cultures contained few living cells, most of which were macrophages of great size. The nuclei in these greatly hypertrophied phagocytic cells were relatively small and crowded to one side. Very few spindle-shaped or elongated polyblasts remained. The cultures were heavily speckled with pyknotic nuclei of dying cells. The red blood cells had been ingested or hemolyzed.

In summary, the following findings were significant:

1. On the basis of the study of the dry smears of the blood, the case was diagnosed as myeloblastic leukemia, and the postmortem observations supported this diagnosis.

2. In the sections of the cultures of the buffy coat, it was impossible to differentiate the large majority of cells from lymphocytes.

3. In the young cultures, some of these lymphoid cells hypertrophied and developed granular cytoplasm; they were then classified as myelocytes.

4. Large numbers of the lymphoid cells developed into polyblasts.

5. In the zone of migration of the early cultures, the cytoplasm of many cells, otherwise identical with the ameboid polyblasts, contained granules indistinguishable from specific neutrophilic granules. These cells were interpreted as being ameboid neutrophilic myelocytes. In the older cultures these cells were absent.

6. In the older cultures it was evident that the polyblast had developed into fibroblast-like cells.

7. Within the explant, and among larger numbers of dying leukocytes, the majority of the cells became actively phagocytic and greatly

TABLE 2.—*Blood Counts in Case 3: Acute Myeloblastic Leukemia*

Date	Red Cells	Hemoglobin, per Cent	White Cells	Differential, per Cent						
				Polymorphonuclear Neutrophils	Small Lymphocytes	Medium-Sized Lymphocytes	Transitionals	Myeloblasts	Myelocytes	Eosinophilic Myelocytes
3/10/31	2,840,000	50	104,000	5	15	15	10	6	49	..
3/12/31	2,610,000	45	94,000	3	..	..	..	49	26	2
3/14/31	1,780,000	37	213,000	..	..	..	..	..	..	..
3/16/31	1,530,000	25	149,000	2	14	11	1	29	23	..

hypertrophied. The remaining cells developed large vesicular nuclei and became fibroblast-like.

8. No evidence was found that red cells had developed during the process of culture.

9. The polymorphonuclear cells were actively ameboid and wandered out from the explant, where they began to show evidence of degeneration as early as twelve hours after explantation.

CASE 3.—*Acute Myeloblastic Leukemia.*—R. C., a boy 5 years of age, was admitted to the Evanston Hospital under the care of Dr. H. O. Lussky, on March 10, 1931, in a critical condition. There was a history of pain and swelling in the right elbow one and one-half years previously, with a recurrence after nine months. Seven weeks before entry a croupy cough developed, and a fever of from 100 to 101 F. had been present since that time. Rather severe pain in the lower half of his right leg had developed one week before admission. Physical examination revealed an acutely ill, poorly nourished child, who was extremely pale. The tonsils were very large and edematous, and shotlike generalized lymphadenopathy was present. The spleen and liver were not palpated. The temperature was of septic type. On March 17, 1931, the child died.

The blood cell counts were as shown in table 2.

The clinical diagnosis was myelogenous leukemia.

The anatomic diagnosis as ascertained post mortem by Dr. F. D. Gunn was as follows: high grade hyperplasia of the bone marrow; hypertrophy (moderate) and myeloid metaplasia of the spleen; leukemic infiltration of the liver, kidneys, intestine and myocardium; myeloid metaplasia of the subcutaneous, mediastinal and peripancreatic lymph nodes; multiple small superficial ulcers and macular hemorrhages of the skin; hemorrhage of the gums; hemorrhages of the muscles and fascia of the chest and abdominal wall, endocardium, myocardium, pericardium, peritoneum, pleura, thymus and esophagus; hemorrhages into the lumen of the intestine; mucous polyp of the ileocecal valve; high grade general anemia. Final diagnosis: acute myeloblastic leukemia.

Observations in Leukocyte Cultures: On the day the cultures were made, the blood cell counts were: red, 1,530,000, with hemoglobin, 25 per cent; white, 149,000; differential (on dry smear stained with May-Grünwald-Giemsa), 9.5 per cent myeloblasts, 60 per cent promyelocytes, 7.5 per cent neutrophilic myelocytes, 3.5 per cent metamyelocytes, 1 per cent polymorphonuclear neutrophils, 16 per cent small lymphocytes, 2 per cent erythroblasts and 1 per cent normoblasts. On searching the smear, only 2 platelets were seen.

The promyelocytes were very large, being twice the diameter of an erythrocyte, and had a smooth oval or plumpy lobulated nucleus with a fine chromatin arrangement; the separation of chromatin and parachromatin was not distinct, and a narrow rim of bluish-gray cytoplasm was speckled with a few small, bright red granules. The myelocytes were similar, except that the nucleus was more frequently round, and there was a dense accumulation of neutrophilic granules at one side of the cytoplasm. The myeloblasts could not be sharply distinguished from the promyelocytes, and were similar except for the absence of granules. The metamyelocytes had fine neutrophilic granules as had also the rare polymorphonuclear cell that was present. Rarely a basophilic erythroblast, a very large, deeply basophilic cell, round in outline and having a large nucleus characterized by a heavy chromatin pattern, was seen. Smaller erythroblasts with polychromatic cytoplasm and typical normoblasts were rare. Small mononuclear cells, the size of an erythrocyte, with a heavy chromatin pattern in the nucleus and blue cytoplasm, were lymphocytes.

In the control cultures, fixed immediately after explantation, the majority of the cells were myelocytes. The nucleus in many of them was deeply lobulated; in others, it was smoothly oval, and fine neutrophilic granules could be seen in the cytoplasm. Few polymorphonuclear neutrophils were seen. Many large, round cells with oval or indented nuclei and basophilic cytoplasm were probably those that were called myeloblasts in the dry smear, and those that were smaller with narrow rims of basophilic cytoplasm were indistinguishable in section from small lymphocytes. No normoblasts were seen.

After two hours of incubation, active ameboid migration had occurred. A relatively wide ring of ameboid forms surrounding the explant consisted chiefly of elongated cells with finger-like pseudopodia. The nuclei in these cells presented bizarre shapes: some were elongated; others were dumb-bell-shaped, spindle-shaped, or deeply wrinkled. The cytoplasm was lightly basophilic. These cells were typical of the early stages in the development of polyblasts. A few polymorphonuclear neutrophils were present in this zone. Within the explant, some of the cells had not changed; others were apparently dying, having deeply pyknotic nuclei, and many were definitely hypertrophied.

After five hours, the ameboid cells in the zone of migration were larger, although there was a higher percentage of rounded cells than in the two hour cultures, especially adjacent to the explant. The ameboid cells had either oval or rounded, deeply lobulated nuclei, and abundant pale grayish-blue cytoplasm containing a few almost imperceptible red granules. In the cytoplasm of a few of the elongated, and in most of the rounded, cells were many dull red granules, which varied in size from those at the limit of visibility, to definite, easily distinguishable ones. A few cells with large, deeply indented or fissured nuclei were present; these, too, had a few scattered fine red granules of a variety of sizes in the cytoplasm. There were very few phagocytic cells in the zone of migration, and in each of these the single, crescent-shaped nucleus was crowded to one side by large vacuoles in the cytoplasm, which also contained small granules. Many small cells with pyknotic nuclei were scattered among the ameboid cells.

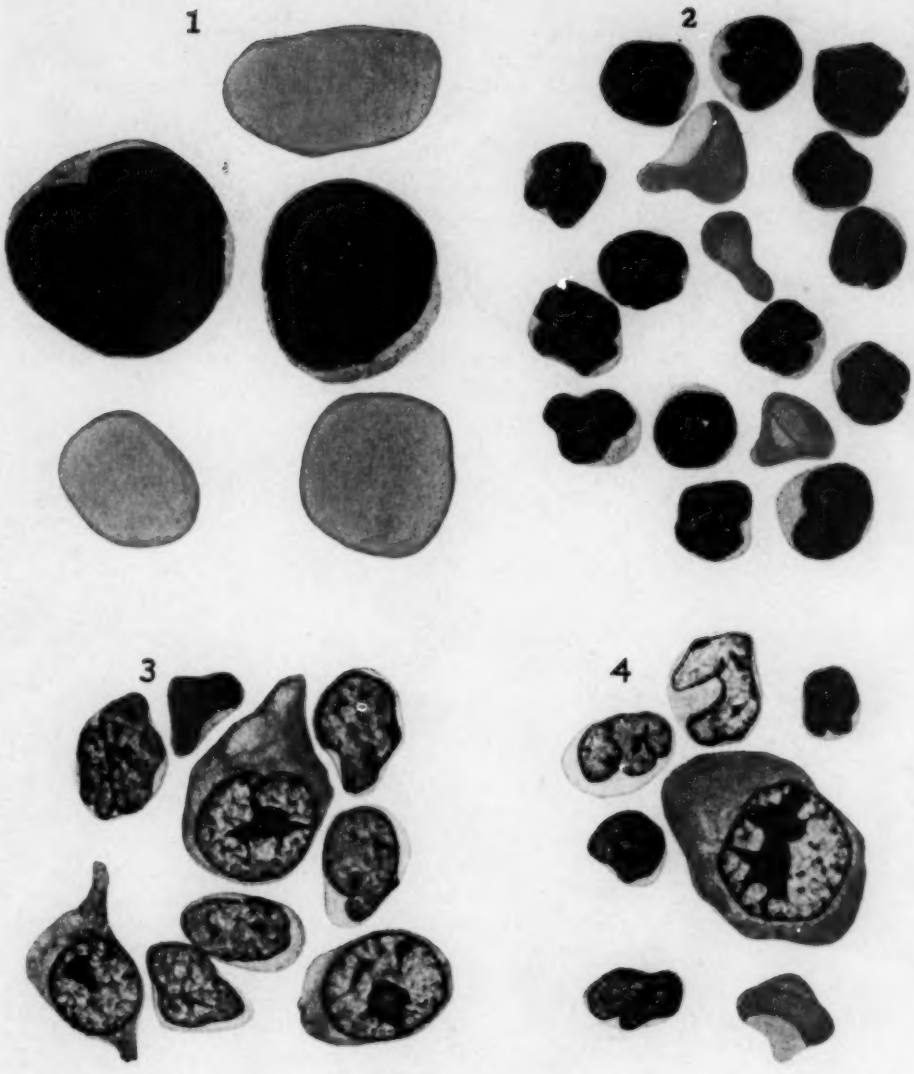
Within the explant, the living leukocytes were rather sparsely distributed among erythrocytes and cells with deeply pyknotic nuclei. Practically all of these living cells were larger than those in the control, or two hour, cultures. Many phagocytes containing ingested cellular débris and many myelocytes with round or deeply lobulated nuclei were present. A few myeloblasts were still to be seen. Mitotic figures were scarce, and an occasional cell with two small, round nuclei was seen.

After eighteen hours, migration had continued, and the ameboid cells presented a great variety of shapes and sizes. In some fields the majority were greatly elongated, with long, slender, finely granular cytoplasmic processes, and spindle-shaped or dumb-bell-shaped nuclei. In other fields more of them were rounded ameboid cells with the polyblast type of nucleus and abundant, irregularly outlined cytoplasm. Several polymorphonuclear cells with lobulated and constricted nuclei and acidophilic cytoplasm were seen. A few of the ameboid cells were phagocytic and contained ingested red blood cells. Within the explant, the leukocytes were scattered through the dense collection of red cells. Most of the hypertrophied cells and some of the smaller cells were phagocytes, which contained digested débris. The rest were myelocytes and myeloblasts. A few mitotic figures were seen. No erythropoiesis was found.

After twenty-nine hours, the zone of migration had widened still farther. In addition to the previously described elongated and rounded, polyblast-like cells, there were many large, round myelocytes, each having a large, round or oval, deeply stained nucleus and slightly acidophilic cytoplasm, in which fine neutrophil granules were present. Many of these cells were also phagocytic, containing ingested erythrocytes and nuclear débris. Occasional mitotic figures were present in the zone of migration. A few dying cells, with acidophilic, round cytoplasm and shrunken, broken or round pyknotic nuclei were seen.

Within the explant, many mitotic figures and occasional binucleated cells were seen. Active phagocytosis by mononuclear cells was occurring; most of these cells had granules in their cytoplasm. Many very large, round cells, some of which had nongranular, basophilic cytoplasm, and others of which had slightly acidophilic, definitely granular cytoplasm, were present. Polymorphonuclear cells were rare within the explant. A large number of small cells had pyknotic nuclei.

After fifty-four hours, most of the cells in the zone of migration were rounded, and many cells were dying. A few large myelocytes with large, oval nuclei and granular, acidophilic cytoplasm were still present. Active phagocytosis by spherical cells with relatively small, crescent-shaped nuclei and a large quantity of vacuolated cytoplasm was taking place; granules were inconstantly present in these cells. These phagocytic cells had not reached the size of those at the same stage of cultivation in case 2. A few polymorphonuclear cells were still present.



EXPLANATION OF PLATE

All the figures are drawn at the same magnification (Zeiss 2 mm., apochromatic objective, 20 × ocular) with camera lucida at microscope stage level.

Fig. 1.—Two lymphocytes from the peripheral blood just before explantation. Dry smear, stained with May-Grünwald-Giemsa stain.

Fig. 2.—A portion of a section through the buffy coat of the centrifugated blood used for explantation, showing the size and homogeneity of the cells.

Fig. 3.—A section through a twenty-four hour culture, showing an apparently unchanged small lymphocyte, several medium-sized and one very large lymphocyte.

Fig. 4.—A similar section showing three unchanged lymphocytes, two monocytoid cells and one exceedingly large lymphocyte with very prominent acidophil nucleolus.

Figures 2, 3 and 4 are from preparations fixed in solution of formaldehyde plus Zenker's fluid and stained with hematoxylin-eosin-azure.

In summary of the morphologic changes observed in the cultures of these cells from myeloid leukemia, in which the large majority were myeloblasts and promyelocytes, the following points were significant:

1. In the earliest cultures, the explant was surrounded by a wide zone of ameboid cells, which were morphologically identical with polyblasts.

2. Shortly afterward, many of these actively phagocytic polyblasts contained numerous reddish-purple granules, which varied in size from that at the limit of visibility to that of eosinophilic granules.

3. Some myelocytes contained ingested cellular débris.

4. Since a large proportion of the cells in the early cultures had granular cytoplasm, it was impossible to say definitely that there had been maturation of myeloblasts into myelocytes, although this seemed probable.

5. The majority of the cells in the cultures were actively ameboid and phagocytic.

6. The nature of the granules, which resembled neutrophilic granules, except for their variation in size, is debatable. It is possible that they were similar to the so-called toxic granulation of the neutrophilic leukocytes during infections.

CASE 4.—*Acute myeloblastic (?) Leukemia.*—W. P., a man 33 years of age, entered the Billings Hospital, March 18, 1931, with the history of a rather insidious onset of headache, oliguria, lumbar pain and constipation ten weeks previously. About three weeks later, he noticed swellings on each side of his neck, which were painless and grew progressively larger. Some drainage had occurred from the one under his ear. Ten years previously, following an attack of measles, he had had swollen glands in the neck, which persisted for about six weeks. Seven years later, three nodules developed in the submental region, which subsided after nonspecific treatment with arsphenamine.

The essential findings on physical examination were those of an acutely ill young adult who was markedly anemic and feverish. There were two draining sinuses on each side of the neck and two just to the right of the midline. The neck was obviously deformed by the subcutaneous swellings. On palpation, several discrete nodules, ranging in size from that of a pea to that of a goose egg, were felt in the chain of lymph nodes posterior to the sternocleidomastoid muscles, as well as many bean-sized nodes in the supraclavicular spaces. The lymph nodes in the axillary, epitrochlear and inguinal regions were palpable also. There was evidence of the presence of fluid in the pleural cavities. The spleen was palpable 8 cm., and the liver 4 cm., below the respective costal margins.

The illness ran a febrile course with peaks of fever of 104 F. for a few days, and then the temperature lowered gradually. The contents of the pleural cavities were aspirated several times for relief of dyspnea, the fluid being sterile each time and showing a cellular content similar to that of the blood. Nodules in the sternum suggesting metastases developed, but this interpretation was not borne out by the roentgen findings. The patient was discharged on May 2, 1931, and died two months later. No autopsy was done.

The blood findings of Dr. Ernestine Kandel, the attending hematologist, are given in table 3.

Dr. Kandel felt that the large number of immature forms seen during the acute febrile stage following admission were myeloblasts, and the diagnosis of acute myeloblastic leukemia was made. As the acute symptoms subsided, the immature forms appeared to be more typical of lymphocytes. She then felt that the condition was chronic lymphatic leukemia with a myeloid reaction during the febrile stage.

Observations in Leukocyte Cultures: On the day of obtaining the blood for culture, March 24, 1931, the cell counts were: red, 1;480,000; hemoglobin, 32 (Dare); white, 250,000; differential (300 cells), 85.3 per cent myeloblasts, 3 per cent metamyelocytes, 3.6 per cent polymorphonuclear neutrophils, 1 per cent promyelocytes, 1 per cent neutrophilic myelocytes, 0.5 per cent eosinophilic myelocytes, 5 per cent small lymphocytes, 0.3 per cent normoblasts and 0.3 per cent mitotic figures (in myeloblasts).

TABLE 3.—*Blood Counts in Case 4: Acute Myeloblastic (?) Leukemia*

				Differential, per Cent								
Date	Hemoglobin, per Cent	Red Cells	White Cells	Neutrophils	Eosinophils	Basophils	Small Lymphocytes	Blasts	Promyelocytes	Neutrophilic Myelocytes	Metamyelocytes	Immature Lymphocytes
3/18	35	1,500,000	300,000	3	..	..	4	84	3	..	..	6
3/24	32	1,400,000	250,000	3.6	..	..	5	85	1	1.5	3	..
3/30	32	1,480,000	320,000	..	..	..	1.5	76.5	5	4	4.5	8.5
3/31	..	.....	330,000	14	1	..	22	27	..	1	6	19
4/ 6	30	1,500,000	280,000	5	..	..	31	36	1	1	2	24
4/15	30	1,100,000	270,000	20	1	..	54	13	..	1	5	6
4/20	30	1,400,000	300,000	..	..	..	45	37	10	3	..	5
4/28	..	.....	.....	15	..	1	23	21	2	..	4	34
5/ 1	..	.....	.....	10	..	..	64	2	..	..	..	24

The myeloblasts in dry smears were round cells about one and a half times as large as an erythrocyte, each with a round or oval nucleus containing from one to three nucleoli and a fine chromatin pattern. The nuclear membrane was thin but distinct. In most of these cells, the nucleus was slightly eccentric and was surrounded by a halo, wider on one side, in which azure granules were prominent. In a few of these cells, fine, almost imperceptible neutrophilic granules could also be seen in this halo. The cytoplasm beyond the halo was basophilic and frequently finely vacuolar. The neutrophilic myelocytes had a round or slightly oval nucleus; the cytoplasm was filled with very fine neutrophilic granules together with a few scattered, more prominent, azure bodies. The metamyelocytes were characterized by a round or elongated oval nucleus with a heavy chromatin pattern, and the presence of specific neutrophilic or eosinophilic granules in the cytoplasm. The small lymphocytes had a small, oval nucleus with a heavy chromatin pattern and a pale blue rim of cytoplasm, in which were seen a few bright azure granules. The normoblasts seen in the smear were typical, with polychromatic cytoplasm and pyknotic nuclei. Occasional mitotic figures were found among the myeloblasts.

The control sections of the buffy coat, fixed at the time of explantation, consisted of a mass of leukocytes with small clumps of erythrocytes scattered throughout the clot. Although there was a fairly equal distribution of the various cells

throughout the tissue, in some areas cells of one type were seen in groups. This was especially true of the erythrocytes and of the polymorphonuclear cells, while the mononuclears were more evenly distributed throughout the sections. The specimen was made up for the most part of large lymphoid cells, varying in size from that of an erythrocyte to twice that size. The nuclei were large, oval or indented, occasionally horseshoe-shaped, with from one to three large nucleoli, a fine chromatin pattern and a thin, but distinct nuclear membrane. The cytoplasm was basophilic. There were frequent cells in which there was a relatively large amount of slightly acidophilic cytoplasm containing varying numbers of fine neutrophilic granules, and a round or horseshoe-shaped nucleus. Frequent eosinophilic cells, most of which were myelocytes, were prominent because of their bright red granules. Occasional ameboid figures were present. Relatively few polymorphonuclear cells were seen among the massed round cells, although in some areas they were numerous. A few smaller cells in which the nuclei stained deeply and had a heavy chromatin pattern were interpreted as being small lymphocytes. Very few normoblasts with polychromatic cytoplasm and round nuclei were seen.

After seven and a half hours, there was a wide zone of migration, the outer edge of which was made up almost entirely of polymorphonuclear neutrophils. Nearer the explant, rounded myelocytes were scattered among the polymorphonuclear leukocytes. Here, too, were seen elongated cells, each with finger-like processes of finely granular cytoplasm and an oval or triangular nucleus containing from one to three nucleoli at one pole of the cell. Frequent, large, round cells with large, horseshoe-shaped nuclei and basophilic cytoplasm resembled monocytes. Occasional eosinophilic leukocytes with round, indented or lobulated nuclei were present. Within the explant, the changes were less marked, but hypertrophy of the majority of the cells had occurred. Their cytoplasm had increased in amount, and in many the nucleus had assumed a slightly eccentric position. While most of the cells appeared larger than in the control tissue, there were also many apparently unchanged lymphoid cells.

After twelve hours, the zone of migration was wider, and there was more variation in the cell forms. The outer edge of the zone was made up chiefly of polymorphonuclear neutrophils. Nearer the explant the number of rounded and elongated cells with granular cytoplasm and oval or kidney-shaped nuclei had increased greatly. Occasional very long, slender ameboid cells with basophilic cytoplasm in which fine granules were distinguishable were seen at the edge of the explant. Frequent eosinophilic myelocytes were present. An occasional medium-sized hemocytoblast with a large, plump nucleus containing from one to three nucleoli outlined by a distinct nuclear membrane and surrounded by a rather wide border of deeply basophilic cytoplasm lay among the ameboid cells.

Within the explant, frequent eosinophilic myelocytes were recognized among the closely packed leukocytes, among which were many cells with finely granular cytoplasm, identified as neutrophilic myelocytes. Rare mitotic figures were seen, and some of the cells were dead.

After twenty-four hours, many of the cells had died. At the outer edge of the zone of migration the polymorphonuclear neutrophils were still the predominating type, although some of them had pyknotic and broken nuclei. Nearer the explant were large numbers of round cells, the majority of which had vesicular nuclei, containing one or more large oxyphilic nucleoli, and narrow rims of nongranular basophilic cytoplasm. Scattered among them were numerous monocytoid cells with deeply indented nuclei and large amounts of nongranular cytoplasm. There were also many round cells with eccentric nuclei and finely granular cyto-

plasm, which were probably metamyelocytes; some of these had band-shaped nuclei and were ameboid. A few elongated cells with nongranular cytoplasm were also present.

At the edge of and within the explant, the majority of the cells had each a large nucleus with from one to two prominent nucleoli and a distinct, although thin, nuclear membrane surrounded by a narrow rim of nongranular basophilic cytoplasm. However, in many cells of this type, fine reddish-purple granules were seen within the cytoplasm. A few cells of the hemocytoblast type were present at the edge of and within the explant. Frequent eosinophilic myelocytes and metamyelocytes were scattered throughout the culture. A few mitotic figures were seen. An occasional phagocytic mononuclear cell, the cytoplasm of which contained ingested débris and the nucleus of which was crowded to one side, was found within the explant.

After thirty-one hours, the zone of migration was still made up chiefly of polymorphonuclear neutrophils. Near the explant lay more of the rounded and elongated myelocytes than at twenty-four hours. The cells tended to be arranged in clumps, in which the polymorphonuclear leukocyte was the predominating type, together with one or two very large, round cells with vesicular nuclei and basophilic nongranular cytoplasm and a few neutrophilic myelocytes and metamyelocytes. No mitotic figures were seen in these groups of cells. Occasional phagocytes with nongranular cytoplasm containing particles of débris were present among the dying cells at the edge of and within the explant.

Within the explant, the large majority of the cells were round. They were larger than the ones in the original explant, and the number of cells with finely granular cytoplasm was greater. There were many transitional forms between the nongranular, round cells, myeloblasts, finely granular neutrophils, myelocytes, metamyelocytes and polymorphonuclear cells. No mitotic figures were seen in the explant.

After fifty-six hours, in the zone of migration many of the polymorphonuclear cells were dead; a few were living, especially in the area near the explant. In this region were many transitional forms, myelocytes, metamyelocytes, band-shaped polymorphonuclears and their lobulated nuclei. Frequent elongated cells with round nuclei and irregular, granular cytoplasm were seen.

Within the explant, the number of phagocytic cells had increased greatly. These active macrophages were large and nongranular, and contained varying amounts of cellular débris. Within the cytoplasm of some were seen engulfed eosinophils and smaller lymphoid cells, the nuclei of which still maintained their chromatin markings and nucleoli. The majority of the living cells in the cultures were definitely larger than those originally explanted. Many cells of the hemocytoblast type were seen; these were large cells from two to three times the diameter of adjacent erythrocytes, and each had a large, oval or kidney-shaped nucleus with a large clump of chromatin and an abundance of deeply basophilic cytoplasm. They were not as large as the hemocytoblasts found in case 1, however. Although the majority of the cells in the explant were lymphoid in type, there were frequent transitional forms to be seen, from the neutrophilic myelocytes, metamyelocytes and polymorphonuclear cells, and a smaller number of similar eosinophilic cells.

After seventy-two hours, large numbers of the cells had died, but the majority were living. In the zone of migration a few long, spindle-shaped cells with greatly elongated processes were seen, in addition to the ameboid myelocytes and polymorphonuclear neutrophils. Within the explant were many eosinophilic and neutrophilic myelocytes and metamyelocytes. A few mitotic figures were seen in these cells. Many nongranular phagocytes were present. Although there were some cells that had not changed in appearance from the explanted ones, the majority of them had increased in size.

In summary of the findings in this case, in which 85 per cent of the cells in the differential smear were considered to be myeloblasts, it may be said:

1. Some of the myeloblasts seemed to mature into myelocytes and polymorphonuclear leukocytes.

2. Some of the smaller myeloblasts hypertrophied into large ones (hemocytoblasts).

3. Frequent macrophages were seen after twenty-four hours. These contained as many as four erythrocytes.

4. A few long, spindle-shaped or stellate-shaped cells appeared in the fifty-six hour and later cultures.

5. No evidence of erythropoiesis was observed.

The explanted cells behaved, then, in culture like those of myeloid leukemia. The question arises as to whether the cells in the dry smear and the blood in the later course of the disease were myeloblasts rather than lymphoblasts.

CASE 5.—*Chronic Lymphatic Leukemia.*—H. I., a white man, aged 60, entered the Billings Medical Clinics with a complaint of tinnitus. On physical examination, generalized lymphadenopathy and splenomegaly were found. He is still under observation. His general condition is fairly good. The clinical course and findings, as well as the blood picture, are those of chronic lymphatic leukemia.

Observations in Leukocyte Cultures: At the time of removal of the blood for culture, the cell counts were: red, 4,440,000, with hemoglobin 13 per cent; white, 219,000; differential, 1.5 per cent polymorphonuclear leukocytes, 86.5 per cent small lymphocytes and 12.5 per cent medium-sized lymphocytes.

In the control culture, fixed shortly after explantation, the explant was seen to be a dense mass of small lymphocytes, with a typical heavy chromatin pattern in the oval nuclei and basophilic cytoplasm. A few polymorphonuclear cells were sprinkled sparsely through the culture. At the edge of the explant were a few cells each of which had a greater amount of cytoplasm and a slight indentation of the nucleus. No monocytes were seen.

After four hours, there had been little if any change in the appearance of the cultures other than an occasional round small lymphocyte which had migrated from the explant.

After sixteen hours, there were large numbers of small lymphocytes in the zone of migration which had maintained their round shape and also the normal nucleus-cytoplasm relationship. A very few monocytoid cells with abundant cytoplasm and an occasional medium-sized lymphocyte were present. A few polymorphonuclear neutrophils and eosinophils were seen. Within the explant there had been little change.

After twenty-four hours, the zone of migration contained a large number of cells. In some of the cultures, the cells in this zone were chiefly small lymphocytes and large monocytes with an occasional polyblast; in other cultures, the zone of migration contained large numbers of dumb-bell-shaped and elongated polyblasts with blunt, long pseudopodia and faintly basophilic cytoplasm, in addition to small lymphocytes and rounded polyblasts. A few large cells with small, round or oval

nuclei and finely vacuolated cytoplasm were seen both in the zone of migration and at the edge of the culture. Some of these contained ingested débris. Many of the cells in the cultures were dying and had pyknotic and broken nuclei.

After seventy-two hours, most of the cells were dying. In the zone of migration there were many ameboid forms, and some of the cells were greatly elongated. Many small and a few large macrophages were present. There were many unchanged cells still present in the explant.

In summary, the changes observed in the cultures of the cells of this case of chronic lymphatic leukemia were:

1. Many of the lymphocytes disintegrated very early, although most of them remained unchanged.
2. Some lymphocytes migrated and developed into rounded and elongated polyblasts, which later became phagocytic.
3. A few large monocytes were seen.
4. No mitotic figures were seen in the cultures.
5. No hemocytoblasts were found.

CASE 6.—*Chronic Myelogenous Leukemia.*—S. D., a Negro, aged 18, was admitted to the Cook County Hospital on Nov. 12, 1930, with a history of insidious onset of loss of weight, dyspnea, enlargement of the abdomen, recent epistaxis, difficulty in hearing and pain in the back. On entrance examination there were evidences of fluid in the chest, palpable lymph nodes, an enlarged spleen, and hemorrhages from the mucous surfaces. The red count was 2,300,000; the total white count, 453,000, with a large number of myelocytes and polymorphonuclear cells. A typical leukemic retinitis developed. The patient suffered repeated hemorrhages from the mucous surfaces. Against advice, on March 24, 1931, he was taken from the hospital.

Observations in Leukocyte Cultures: At the time of obtaining the blood for cultures, the cell counts were: red, 2,300,000, with hemoglobin 50 per cent; white, 453,000; differential, 4 per cent band-shaped and 27 per cent segmented polymorphonuclear leukocytes, 4.5 per cent myeloblasts, 52 per cent neutrophilic myelocytes, 3 per cent eosinophilic metamyelocytes, 3 per cent myelocytes, 2 per cent micromyelocytes, 3.5 per cent normoblasts, 1 per cent basophilic erythroblasts, 0.5 per cent basophil leukocytes and no lymphocytes.

In the dry smear, the neutrophilic myelocytes were large cells, ranging from one and one-half to two and one-half times the size of an erythrocyte. The nuclei were large and had an almost imperceptible nuclear membrane and a fine chromatin network, and in the more deeply stained cells, from one to two nucleoli. The cytoplasm was filled with very fine neutrophilic granules. In most of these cells azure bodies were also present. The myeloblasts were similar large cells with faintly basophilic, reticular cytoplasm without neutrophilic granules, although azure granules were constantly present. The eosinophilic myelocytes and metamyelocytes had the characteristic large, red granules packed closely in the cytoplasm. The micromyelocytes were the size of an erythrocyte, had an oval nucleus, fine neutrophilic granules in the cytoplasm, and large, azure bodies sprinkled throughout the cytoplasm. The normoblasts had a heavy chromatin arrangement in the small, round nucleus, and an irregular, smooth, reddish-yellow cytoplasm. The basophilic erythroblasts were about one to one-half times as large as an erythrocyte and had a deeply basophilic cytoplasm and a large nucleus, which had a fine chromatin arrangement and usually one nucleolus. The basophilic leukocytes had the characteristic large, bluish-purple granules in the cytoplasm.

In the control sections of the buffy coat, the large majority of the cells were only slightly larger than an erythrocyte, being rounded and not flattened as in the smear. The nuclei were round or oval, had each a definite nuclear membrane, fine chromatin arrangement and from one to three nucleoli. The cytoplasm in most of the cells was acidophilic and vacuolar, and fine neutrophilic granules were seen with difficulty. A few cells were larger, but otherwise similar. Many eosinophilic myelocytes were prominent because of their large, red granules. Many polymorphonuclear cells were scattered among the round cells.

After six hours, the explant was surrounded by a wide zone of migration, made up of neutrophilic, and fewer eosinophilic, polymorphonuclear leukocytes. The migrating cells had reached the edge of the clot and were crowded closely together there. Within the explant most of the cells were larger than those in the control explant. Most of them were neutrophilic myelocytes, among which a few mitotic figures were seen. A few polymorphonuclear neutrophils were present. A few normoblasts with irregularly polychromatic cytoplasm and shrunken, round, pyknotic nuclei were easily recognized among the leukocytes.

After twenty-one hours, the zone of migration was still composed chiefly of polymorphonuclear neutrophils and eosinophils, especially at the periphery of the zone. Nearer the explant were many large myelocytes, the cytoplasm of which was often vacuolated, and the nuclei of which were large and round or oval, contained from one to three nucleoli, and had a fine chromatin pattern with a distinct nuclear membrane. More of the elongated myelocytes were present than in the six hour cultures. Frequent metamyelocytes with band-shaped or round nuclei and granular cytoplasm were seen scattered among these round cells. Within the explant there had been hypertrophy of many of the cells. The cytoplasm had increased in amount and was finely granular, and many of the cells had plump horseshoe-shaped or deeply bilaterally indented nuclei. Fewer mitotic figures and few normoblasts were seen here than in the earlier cultures.

After thirty and forty-five hours, the number of myelocytes and metamyelocytes in the zone of migration had increased further. It was possible to find all intermediate forms between the smaller myelocytes in the explant and the larger myelocytes and metamyelocytes in the migration zone. Within the explant, the majority of the cells were myelocytes. A few mitotic figures were present. More metamyelocytes and polymorphonuclear cells were present than in the twenty-one or thirty hour cultures. A few normoblasts could be distinguished from the dying myelocytes by their irregularly shaped, polychromatic cytoplasm and round, pyknotic nucleus. An occasional cell contained small particles of phagocytosed débris. After three days, the cultures showed evidence of death of many of the cells. In the zone of migration, polymorphonuclear cells and round metamyelocytes were in great predominance, while few ameboid myelocytes were seen. Within the explant, the majority of the cells were myelocytes, but large numbers of metamyelocytes and many polymorphonuclear cells were present. No normoblasts were identified; many dying cells were seen. After four days, the cultures contained few living cells, most of which were metamyelocytes and polymorphonuclear leukocytes. A very rare round macrophage was seen. Shadows of cells and cells with pyknotic nuclei were frequent.

In summary, the following observations were made:

1. Evidence was found that myelocytes mature in culture into metamyelocytes and polymorphonuclear cells.

2. No evidence of erythropoiesis was present.

3. Very few phagocytic cells developed in these cultures, which consisted for the most part of myelocytes and polymorphonuclear cells.

CASE 7.—*Chronic Myelogenous Leukemia.*—J. B., a man 50 years of age, entered Michael Reese Hospital on Nov. 12, 1931, under the care of Dr. H. Bunswanger, with a complaint of pleurisy pain of prolonged duration and poor vision. Six months previously, while he was under observation for the eye trouble, a leukemic blood picture was found, the total white cell count being, then, 165,000 with a large number of myelocytes. At the time of entry in November, he was not acutely ill and no lymphadenopathy was present, but the spleen was markedly enlarged. The blood cell counts on Nov. 17, 1930, were: white, 510,000; differential, 10 per cent polymorphonuclear neutrophils, 24 per cent immature leukocytes (band forms), 38 per cent promyelocytes, 9 per cent myeloblasts, 29 per cent myelocytes and 2 per cent normoblasts. He was discharged from the hospital on Nov. 26, 1931, in good condition, with orders to return for roentgen treatment at a later date.

Observations in Leukocyte Cultures: The blood cell counts on the day of culture were: red, 3,900,000, with hemoglobin 70 per cent; white, 165,000; differential, 10 (2.2 per cent) large myeloblasts, 178 (44.5 per cent) small myeloblasts, 64 (16 per cent) small neutrophilic myelocytes, 5 (1.2 per cent) large neutrophilic myelocytes, 19 (4.7 per cent) metamyelocytes, 39 (9.9 per cent) band-shaped, 79 (19.7 per cent) mature polymorphonuclear neutrophils, 3 (0.7 per cent) eosinophilic myelocytes and 3 (0.7 per cent) small lymphocytes.

In the dry smears, the myeloblasts resembled large and medium-sized lymphocytes; the nucleus was difficult to demarcate from the somewhat vacuolar basophilic cytoplasm, had a fine chromatin pattern, and from two to three vague nucleoli. No granules were seen in the cytoplasm. The small myelocyte had a large, bean-shaped nucleus with a more definite nuclear membrane and one to two nucleoli; the cytoplasm was slightly acidophilic, and fine neutrophilic granules were present on the side of the indentation of the nucleus. In the larger myelocyte, the nucleoli were more prominent, and scattered throughout the cytoplasm were frequent azure bodies in addition to the fine neutrophilic granules.

The control sections of the buffy coat consisted for the most part of mononuclear cells, the greatest number of which were about one and a half times the size of an erythrocyte; each had basophilic cytoplasm and a large nucleus with a fine chromatin network, a distinct nuclear membrane and from one to three nucleoli. These were the cells that were called myeloblasts in the differential dry smear. There were also large numbers of cells with similar nuclei, frequently eccentrically placed, in the cytoplasm of which fine neutrophilic granules were visible, and these were recognized as neutrophilic myelocytes. A few eosinophilic myelocytes and metamyelocytes were present. Frequent neutrophilic metamyelocytes and band-shaped and mature polymorphonuclear cells were scattered throughout the tissue. A rare cell, with a round pyknotic nucleus and an irregular polychromatic cytoplasm, was identified as an erythroblast. There were a few large, round cells, each with a nucleus containing two or three nucleoli and a heavy nuclear membrane, in which no granules were seen in the slightly basophilic cytoplasm.

After two hours' incubation, the culture was surrounded by a zone of migrating cells, which was made up for the most part of polymorphonuclear neutrophils. Near the explant were numerous neutrophilic myelocytes. Within the explant there had been little change in the cells since explantation.

After ten hours, the zone of migration had increased greatly in diameter. While the majority of the cells were ameboid polymorphonuclear neutrophils, there were also seen similar cells in which the nucleus was not lobulated, but round, wrinkled or oval. Near the explant were many round myelocytes which had wandered from

the explant. Within the explant, the majority of the cells were recognized as myelocytes, having a round or oval nucleus containing from one to three nucleoli, fine clumps of chromatin, and a distinct nuclear membrane surrounded by a finely granular cytoplasm. A few large cells with similar nuclei and nongranular cytoplasm were seen. These resembled large lymphocytes, but were probably myeloblasts. Several metamyelocytes and eosinophils were scattered throughout the explant. No erythroblasts were recognized.

After twenty-four hours, the cells at the periphery of the zone of migration were polymorphonuclear leukocytes and myelocytes. Near the explant were many metamyelocytes, large myelocytes, smaller myelocytes and a few round cells with nongranular cytoplasm. Within the explant, the majority of the cells were myelocytes. Many of these were large and had a relatively greater amount of cytoplasm than those in the earlier cultures. There were a few hemocytoblasts with a large, oval nucleus, containing two or three nucleoli, and basophilic cytoplasm.

In several places in the culture were groups of normoblasts with round, pyknotic nuclei and irregular orthochromatic cytoplasm. A few dying leukocytes were seen, which closely resembled these normoblasts. However, these cells had a round outline and definite acidophilic cytoplasm; their nuclei were of various sizes and shapes, and nuclear débris was present within the cytoplasm. The similarity of dying cells to normoblasts makes it necessary to be guarded in the identification of a cell as a normoblast in blood cultures. Only when the various stages in erythropoiesis could be demonstrated was it considered prudent to identify the cells as such.

After twenty-six hours, the largest number of cells in the zone of migration were ameboid neutrophilic myelocytes and polymorphonuclear neutrophils. There were groups of cells, more frequent at the periphery of this zone, in which there were many transitional forms between the ameboid myelocytes and the polymorphonuclear neutrophils. Within the explant, the majority of the cells were granular; a few were polymorphonuclear, and a few had the horseshoe-shaped nucleus of a metamyelocyte. There were a few mitotic figures in large, round cells in the cytoplasm in which were dull red granules larger than the granules in the majority of the neutrophilic myelocytes and resembling those described in case 3. These granules were also seen in a few of the smaller round cells. A few dying leukocytes, but no normoblasts, were seen in these cultures.

After forty-eight hours, the type of cells in the zone of migration had not changed, but here the transition from myelocytes to polymorphonuclear cells was clearly demonstrated by the presence of a zone of myelocytes immediately surrounding the explant, which merged into a zone where most of the cells were myelocytes, and into a peripheral zone where the largest number of polymorphonuclear cells were seen. No mitotic figures were seen in the zone of migration. Within the explant, the majority of the cells were round myelocytes. A few eosinophilic myelocytes were prominent because of their large, red granules. A few mitotic figures and macrophages were seen.

After three days, many of the cells within the explant and also in the zone of migration were disintegrating. In the polymorphonuclear cells and in the elongated, mononuclear, granular cells, the granules were larger than those in the previous culture and were identical with those described in case 3. Within the explant most of the living cells were large myelocytes containing the same dull red granules, and there were frequent metamyelocytes and a few polymorphonuclears scattered throughout the explant.

After five days, most of the cells in the culture had disintegrated. In the zone of migration, most of the cells were rounded, had irregularly shaped, granular

cytoplasm and dark nuclei. Within the explant, most of the cells had pyknotic, shrunken nuclei and acidophilic cytoplasm, in which granules were seen with difficulty. There were many phagocytic cells having a small crescent-shaped nucleus crowded to one side by a large amount of vacuolated cytoplasm containing ingested débris.

In summary, in cultures of the blood in this case of chronic myelogenous leukemia, the following observations were made:

1. The myeloblasts developed into myelocytes, and the latter matured into polymorphonuclear leukocytes.

2. Doubtful evidence was found which indicated that erythrocytes had developed from a large basophil progenitor.

3. Myeloblasts developed into macrophages in the older cultures.

In four cases of aleukemic lymphatic leukemia in children, one of whom had a total white cell count of 14,000 and the other three of less than 5,000, the cultures of the leukocytes proved unsatisfactory. The cells did not grow well. The lymphocytes disintegrated early, and although in all of the cases a few phagocytes were obtained in the cultures, after thirty-six or forty-eight hours their origin could not be traced.

COMMENT AND SUMMARY

The outstanding morphologic changes in the leukocytes in culture were as follows:

1. Pronounced ameboid activity of the cells was observed in all cases of lymphatic and myeloid leukemia.

2. Hypertrophy occurred in the great majority of the explanted cells of all the types of leukemia, except chronic lymphatic and aleukemic leukemia, a large number of the cells of which remained unchanged and showed evidence of early degeneration.

3. Monocytoid cells developed in all cases, the fewest being present in the chronic forms of myeloid and lymphatic leukemia, and the greatest number, in the acute forms of both types.

4. Polyblasts or macrophages were found in all cases, the largest number developing in acute myeloblastic leukemia. In the cases in which these cells were found in largest numbers, fibroblast-like cells also were seen in the later stages.

5. Hemocytoblasts were seen to develop in the acute and chronic myeloid leukemias and also in the acute lymphatic leukemia, in which 96 per cent of the cells were small and medium-sized lymphocytes.

6. Evidence of the maturation of myeloblasts into myelocytes was found in the cases of myeloblastic leukemia.

7. Evidence that myelocytes matured into polymorphonuclear leukocytes in cultures was not conclusive.

8. Evidence of the development of members of the red cell series from a basophilic progenitor (hemocytoblast or myeloblast) was incomplete.

9. Granules similar to neutrophilic granules except for their variation in size and shape were seen to develop in two cases of myeloblastic and one case of chronic myeloid leukemia. The nature of these granules was not determined, but they did not develop in the cases of acute, chronic or aleukemic lymphatic leukemia. They differed from the granules of the X cells of Rhoads and Parker in that they were larger and more irregular in size.

In answer to the questions listed in the introduction of this article, it may be said:

1. Leukemic blood cells in culture undergo many of the same changes that normal leukocytes do in similar preparations.

2. In these experiments, the lymphoblast of acute lymphatic leukemia differed in its behavior in culture from the myeloblast of acute myeloid leukemia by not forming myelocytes. Both of these cells produced monocyte-like cells, polyblasts or macrophages, fibroblast-like cells, and hemocytoblasts.

3. The lymphocytes of chronic lymphatic and aleukemic leukemia did not show the active growth and morphologic changes that were observed in those of acute lymphatic leukemia. Those few cells that did hypertrophy, however, behaved like those of the acute condition.

4. Strong evidence that the small lymphocyte is a cell of various potencies was found. The evidence of previous investigators (Timofejewski and Benewolenskaia, 1926; Maximow, 1928; Caffier, 1927; Bloom, 1928) that this cell has the capacity of ameboid activity and of development into monocyte-like cells, polyblasts and fibroblast-like cells is corroborated. The observation in the present investigation that the small lymphocytes produce large lymphocytes (typical hemocytoblasts) in culture is of great interest. This is confirmatory evidence for the contention of Weidenreich (1911), Dominici (1901-1920), Maximow (1907-1927) and Jolly (1923), who considered the small lymphocyte of the blood to be able to develop under certain conditions into the large lymphocyte, a view that is denied by practically all other hematologists, particularly by Nägeli, Schilling and Ferrata, who considered the small lymphocyte to be completely differentiated. According to the unitarian theory of hematopoiesis as advocated by Maximow, Jolly, Weidenreich, Downey, Jordan and others, the lymphocyte has still other developmental potencies, namely, into (1) the macrophage direction and (2) the erythrocytic and myelocytic series of blood cells. But the latter has not been demonstrated for blood lymphocytes in culture, although the lymphocytes of lymphatic tissue explanted with bone marrow extract developed into myelocytes (Maximow, 1923).

5. The observations of Timofejewsky and Benewolenskaja (1926) that the myeloblasts produced granulocytes, monocytes and fibroblast-like cells were corroborated. However, conclusive evidence of erythropoiesis in the cultures was not observed. It is, of course, possible that the absence of this finding may have been due to unfavorable conditions in my cultures.

6. The finding of cells morphologically identical with monocytes as transitional stages in the development of polyblasts in the cultures of leukocytes of all the types of leukemia is contradictory to the theories that the monocyte arises from a specific stem cell. The present understanding of the origin of the monocyte is admittedly one of great confusion, owing to the fact that various theories have been built up without sufficient observations of the origin of the cells in situ (Bloom, 1928). The main theories now actively debated fall into four main groups: 1. Some investigators (Nägeli, 1923) believe that the monocyte originates in the bone marrow from the myeloblast. 2. Others (Cunningham, Sabin and Doan, 1925) assert that the monoblast, normally present in the spleen and connective tissues, is the specific forerunner of the monocyte. 3. Others (Weidenreich, 1911; Maximow, 1927; Bloom, 1928) feel that the monocyte is a modified lymphocyte or hemocytoblast. 4. Others (Aschoff, 1913; Schilling, 1929) contend that the monocyte is a derivative of the histiocyte. 5. Still others (Downey, 1931) ascribe to it an origin from several of these sources. The findings in this investigation substantiate the belief of other investigators using the tissue culture method, namely, that the monocyte can develop into a macrophage, and support the observation of Maximow (1925-1928), Bloom (1928), Caffier (1927), and Timofejewsky and Benewolenskaja (1926) that in the development of a lymphocyte into a macrophage the lymphocyte passes through a monocyte-like stage. This lymphoid percursor is the much discussed cell called by some the lymphoblast and by others the myeloblast, neither of which can be differentiated from the other in sections or dry smears. This investigation strengthens, then, the contention of the unitarian school that the monocyte is a modified lymphocyte. Until it can be more satisfactorily demonstrated that the lymphocyte can be morphologically differentiated from the monoblast by any of the known histologic methods, including supravital stain, and since the "rosette formation" is not specific for the monocyte (Hall, 1930), it appears that the weight of evidence is in favor of the "monoblast" being a lymphocyte or a myeloblast, or both (i. e., a hemocytoblast).

7. As to whether the tissue culture method may be relied on as a diagnostic aid in the determination of the types of leukemia encountered clinically, it may be said that it is of value in those cases in which the majority of the leukocytes of the blood are of the large, undifferentiated type. When it is impossible in dry smears to differentiate the

"myeloid cell" or a "lymphoid cell," and when on growth of leukocytic cultures granular cells are observed to develop, one may conclude that the explanted leukocytes had the potencies commonly recognized as characteristic of myeloid cells. If no granular cells are seen to develop, one may conclude that the cells are of the lymphocytic type.

8. It is not necessary here to review in detail the many theories of the formation of blood, since recent surveys by Jolly (1923), Downey (1928), Maximow (1927), Sabin (1928), Doan (1931) and Bloom (1932) have covered this subject so completely. Suffice it to say that the unitarian view supported by Dominici, Dantschakoff, Weidenreich, Jolly, Maximow, Jordan, Downey, Bloom and others, namely, that the hemocytoblast or large lymphocyte of the blood and lymphatic tissue is endowed with manifold potencies of development, is the theory which gains the most support from this investigation.

The polyphyletic theories supported by Ehrlich, Schilling, Ferrata, Sabin, Cunningham and Doan, Aschoff, Schridde and Nägeli are incompatible with the findings in these cultures. The concept of Sabin, Cunningham and Doan (1925) that vascular endothelium produces the red cell series and the clasmatocyte, while the primitive reticular cell produces the three stem cells, the lymphoblast, the myeloblast and the monoblast, gains no support except of a negative nature, notably the absence of erythropoiesis in my cultures; but Timofejewski and Benewolenskaja (1926) reported this finding. That the lymphoblast was indistinguishable from the myeloblast, and that monocytes, polyblasts and fibroblast-like cells were derived from both, is evidence against the irreversible differentiation of these blast forms.

This investigation supports the concept that a sharp distinction cannot always be drawn between myeloid and lymphatic tissue (Maximow and Bloom, 1930). What determines the types of cellular response of the blood-forming organs in the various clinical types of leukemia and allied conditions, is a matter at present under active inquiry by numerous investigators.

CONCLUSIONS

Detailed accounts are given of the observations made during growth of cultures of leukocytes taken from two patients with chronic myelogenous, one with chronic lymphatic, three with myeloblastic, and one with acute lymphatic, leukemia, the case histories of which are briefly sketched.

In the myeloid and myeloblastic types, myelocytes, polyblasts, fibroblast-like cells, monocytes and hemocytoblasts developed.

In the chronic lymphatic type, a few monocytes, many polyblasts and fibroblast-like cells appeared, although many of the explanted cells remained unchanged.

In the acute lymphatic type, monocytes, polyblasts, fibroblast-like cells and epithelioid cells developed in large numbers, as well as hemocytoblasts, morphologically identical with those found in lymph nodes and bone marrow.

The development of these hemocytoblasts from known small lymphocytes is of hematologic significance, since, according to the unitarian school, in both the embryo and the adult normal body, the free stem cell is a polyvalent cell capable of producing all other types of blood cells.

It is felt that the tissue culture method may prove to be of diagnostic aid in leukemias in which "blast" forms dominate the blood picture.

Dr. William Bloom of the Department of Anatomy of the University of Chicago, in whose laboratory the work was done, gave me his advice and counsel; Miss Ruth Holton, technical assistance.

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ORIGIN OF TEETH IN DERMOID CYSTS

SOME REFLECTIONS ON THE ENIGMA OF THE TERATOMA

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Teratoma, with its curious aggregations of tissues closely resembling those of the developing or adult body, has been a source of wonder to all investigators. The extraordinary degree of differentiation of the tissues with formation of organ-like structures has so enthralled the imagination of observers that they seem to have been unable to attack the problem of these bizarre growths except by way of the view that such collections of tissues actually are malformed organs, or attempts at their formation. The writings on teratoma show a wealth of hypothesis, the plausibility of which has led to superficially apt conclusions. In the middle ages, the opinion prevalent was that the growths were malformed fetuses—a judgment of the Deity on immoral practices—and, as happens when a hypothesis comes to be accepted widely, the suggestion was regarded as fact, and immorality was deduced from the presence of pelvic teratoma. The acceptance of this view was unquestioned until it was strenuously opposed by Blumenthal. The death knell was sounded by Baillie [1] in 1789 when he reported an example of dermoid cyst containing teeth and hair, etc., occurring in the ovary of a virgin girl, aged 11.

Until the end of the last century only sporadic reports and observations were made. Then the subject was attacked not only by gross observation but also by histologic examination and experimental investigation. The same point of view—that the growths must be related in some way to a fetus—manifested itself and controlled investigations.

The cystic tumors, particularly those in the ovary, were thought to be obvious malformed embryos. The protuberance of Rokitansky was said to represent the embryo, and the cyst was regarded either as an amniotic cavity or as a dilated graafian follicle. Gross structures were interpreted as limbs, brain, etc., without adequate investigation of their

1. Baillie, M.: London M. J. **10**:322, 1789.

intimate nature. Histologic examinations resulted in the discovery of a great many tissues grouped together in a more or less orderly manner, and extraordinary conclusions were again drawn, all to fit the hypothesis discussed in a foregoing paragraph. Eyes have been described, as well as brain, trachea, alimentary canal and even such components as an appendix or cecum (Pommer [2]). Examination of structures similar to those referred to or investigation of the original reports and illustrations (when present) causes one to pause in admiration of the fertility of imagination of some of these writers.

Experimental work showed that parthenogenesis of vertebrate ova could be induced in some animals. It was also shown that structures similar to those found in teratoma could be produced by the injection into animals of embryonic tissue. This was all taken as direct evidence, and the case for the heterochthonous development of teratoma seemed to be complete.

The almost invariable presence of nervous tissue and the predominance of structures of ectodermal origin were explained by the hypothesis that intracystic tension prevented the growth of the "fetus" after a time, and that thus those structures which are to be found best developed in the early embryo, namely, ectoderm of the anterior end of the embryo and particularly nervous tissue, are to be found best developed in the tumors. Many difficulties of interpretation arose, since in some cases the structures did not appear to belong to the anterior end of the embryo.

Two pathologists accepted their own observations in preference to the hypothesis and interpreted structures as hind limbs and labia (Shattock [3]) and scrotum (Ingier [4]). This preference is one that needs cultivation particularly when dealing with the teratoma. If one goes so far as to assume, with Shattock, that in some cases the teratoma does not develop in the same way as an embryo—that is, the cell growth is controlled in some way that is quite different from that in which the cell differentiation in the fetus is controlled—it is not difficult to take the further step of realizing that the teratoma possibly does not correspond to a malformed fetus, and that the resemblances are of gross morphologic character and accidental.

Examination of many of the structures that have been "recognized" in these growths shows that purely superficial resemblances are taken as criteria of identity. There is one structure, however, about which there is a great deal of difficulty, namely, a tooth. In all probability, teeth in "dermoids" have been greatly responsible for the development

2. Pommer, G.: Centralbl. f. allg. Path. u. path. Anat. **1**:260, 1890.

3. Shattock, S. G.: Tr. Path. Soc., London **58**:267, 1907.

4. Ingier, A.: Beitr. z. path. Anat. u. z. allg. Path. **43**:356, 1908.

of the current hypothesis. From the presence of teeth, bones have been identified as maxillae and a cavity as a mouth or, when pseudo-stratified ciliated epithelium was present, as a pharynx showing a junction of alimentary and respiratory tracts. A large superstructure of hypothesis has developed around the tooth.

The presence of teeth appears to support the hypothesis of a malformed parthenogenetic fetus more strongly than other observations. Since so many difficulties are found in the application of the hypothesis to other structures, it is desirable to examine the teeth afresh. Are they actually teeth comparable in every respect with oral teeth?

To begin with, however: What is a normal tooth?

A working definition is as follows: Teeth are highly specialized structures of definite morphology and chemical nature, which are organs of mastication and, to some extent, speech, and which develop by an epithelial downgrowth, at the site of the dental groove, from the squamous epithelium of the oral mucosa.

An examination of "dermoid" teeth shows that the first criterion applies accurately; the teeth correspond to those of the mouth in every way; they present enamel, dentin, cement and a pulp chamber. It is true that the enamel may be laid down in irregular masses, and that the pulp chamber may be unusually large and contain débris in the form of pulp stones, but nevertheless the general features of oral teeth are so faithfully reproduced that one is able to distinguish incisor, bicuspid and molar teeth. The enamel and dentin appear to be identical in structure and nature with those of oral teeth (fig. 6).

When we come to the next criterion, however, there is considerable difficulty. A teratoma is not a separate individual; it obtains its nutriment from the blood vessels of the host without the interposition of a placenta, and there is no possiblity of the growth having a separate existence. The development of any tissue in such a growth cannot have purposive significance, even if we were disposed to accept such a hypothesis for development in general. Thus the idea of mastication in a teratoma is almost as great an absurdity as some other suggestions met with in the literature, when taken to their logical conclusions. Why should some growths require three hundred teeth, and why should a tooth occur without any other tissue? Explanations of these phenomena might be found if the teeth were arranged in a "mouth," but they occur in many places, even on the "scalp" of the protuberance. We have seen a single tooth in a small cavity lined by squamous epithelium occurring in a comparatively large growth consisting otherwise entirely of thyroid tissue. It seems futile to say that all of the embryo failed to develop except one tooth and a thyroid gland, which became hyperplastic. It becomes apparent that to main-

tain the hypothesis that the teratoma is an extraneous growth arising from an ovum or from a blastomere, we must superimpose hypothesis on hypothesis.

Thus the conclusion is forced on us that, from this point of view, teeth in a teratoma, though almost identical in structure with those in the mouth, have a different biologic significance; that is, they are not a portion of the mouth of a malformed embryo, but have arisen in tissues of the host by a process of differentiation usually observed only in the region of the mouth.

It is necessary now to examine the mode of development as shown by the relationships of the teeth to surrounding tissues.

Fully formed teeth give little indication of their mode of origin. Their microscopic structure closely resembles that of normal teeth, and they project usually from a surface lined by squamous epithelium and are frequently embedded in bone, though at times only in connective tissue.

They appear thus to fulfil the third criterion of the definition in that they appear to arise from squamous epithelium. It might be argued that the presence of a mouth is merely a question of interpretation, and that, in explanation of teeth on the surface of the protuberance, the mouth of the embryo might be considered to be opened out widely.

Examination of developing teeth in a teratoma completely negatives this view and supports the contention submitted concerning the essential biologic difference of teratomatous teeth from oral teeth.

MATERIAL

One typical specimen of a teratomatous ovarian cyst is taken as illustrating the features of these developing organs.

The cyst was 3½ inches (8.9 cm.) in diameter, contained greasy fluid and had a projection into its cavity from one wall, measuring 2 by 2 by 1½ inches (5 by 5 by 3.7 cm.). This projection contained a large number of small cysts. These cysts were variously lined by different types of lining, and between them there was tissue containing muscle, bone, cartilage and areolar connective tissue.

In that portion of the protuberance near its junction with the wall of the main cyst there were some small masses of bone and a few small teeth. These were in relationship to some of the subsidiary cysts. One such typical cyst will be described.

This cyst, irregularly oval, lay close both to ovarian stroma at the basal attachment of the protuberance and to the surface of the protuberance, being separated from the cavity of the main cyst surrounding the protuberance, in one part, by tissue only one thirty-second of an inch (0.08 cm.) in thickness.

The tooth related to this cyst and the associated bone lay on one aspect of its cavity, and the tooth was entirely embedded in soft areolar tissue. After the cyst was opened, a small depression in the cyst wall near the tooth, could be seen macroscopically. The wall was lined by a smooth, shining tissue and appeared to

be uniform throughout its extent. The tissues concerned were removed, decalcified and examined by serial sections.

Microscopically, the wall showed various appearances. In greater part, the tissue nearest to the lumen of this cyst consisted of material of a structure identical with tissue of the central nervous system. It is proposed to call it neuroglial tissue, but it is to be appreciated that there is no proof of its nature; it is merely morphologically identical with neuroglial tissue (fig. 2). In some parts, this tissue lined the wall of the cyst. In other parts, on the internal aspect of this neuroglial tissue, there was a pseudostratified ciliated epithelium, which in some places became a single layer of columnar cells (fig. 3). The cells had basally situated nuclei, but at times this polarity was reversed, and a clear area of cell projected toward the subepithelial tissue. There was a fibrillar connection between the

Fig. 1.—Drawing showing the relationship of the developing tooth to the neighboring cyst. The cyst is lined for the most part by nervous tissue (fig. 2), but in part there is an epithelial lining (fig. 3), and in the crypt a portion of this has become squamous. A neighboring cyst also lined by nervous tissue is shown on the left. Ovarian stroma is present above.

epithelium and the subjacent tissue, thus increasing the resemblance to nervous tissue with an internal layer of embryonic epithelium.

In the area of the wall near the tooth there was a definite crypt resembling the dental furrow, and from this down toward the tooth could be seen discontinuous strands of connecting epithelial tissue. Beneath the tooth was a small mass of bone.

More thorough examination revealed a number of astonishing (if one still clings to preconceived notions) accompaniments. Beneath the neuroglial tissue, in addition to the bone, there were fat and other mesenchymal derivatives. In some areas where the neuroglial tissue was thin, the pseudostratified epithelium grew down and formed glands of the mucous and serous types (fig. 4). The resemblance to respiratory epithelium in this part was remarkable. In the depths of the crypt, for a short distance the epithelium became squamous (fig. 5). From this epithelium in the

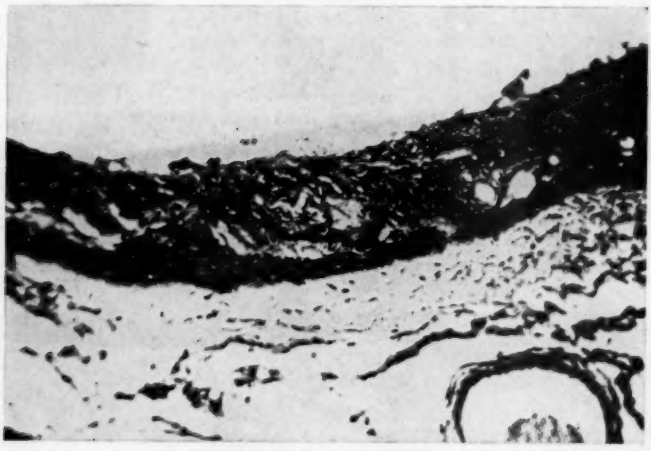

Fig. 2.—Photomicrograph of a portion of the wall of the cyst showing tissue that morphologically is indistinguishable from nervous tissue; × 90.

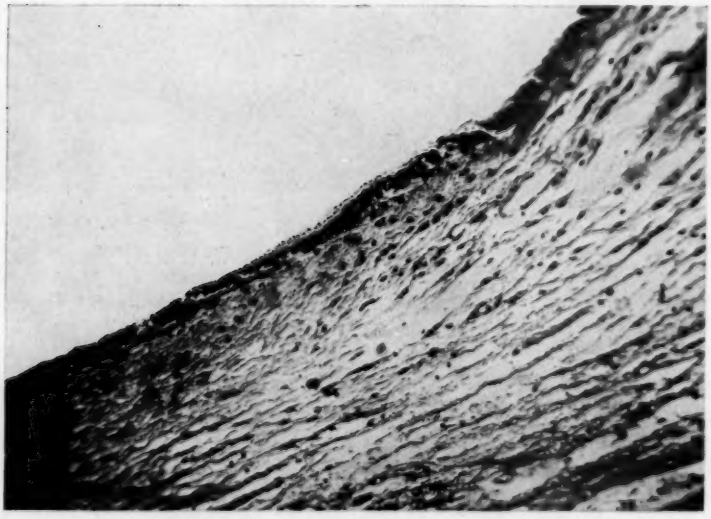

Fig. 3.—Another portion of the cyst, in which ciliated epithelium overlies the nervous tissue; × 90.

Fig. 4.—Another portion continuous with that shown in figure 3, in which glands have developed; × 40.

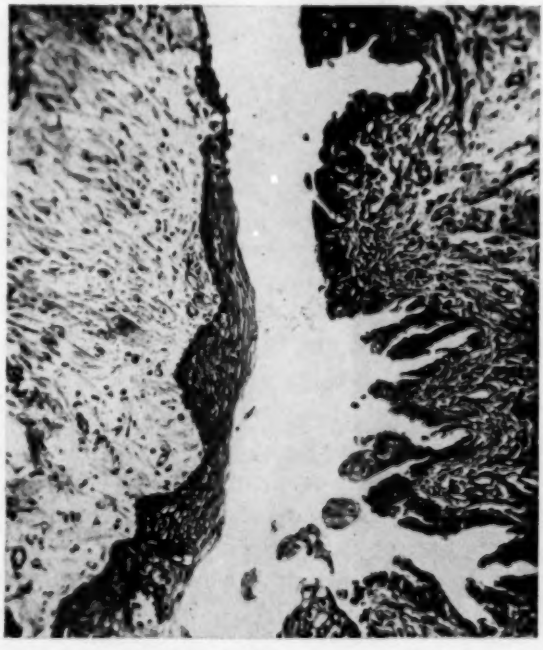

Fig. 5.—The epithelium of the crypt—on one side squamous; × 90.

crypt to the region of the "root" of the tooth there were in the tissue, collections of epithelial cells in strands and groups that imitated closely the epithelial débris of Malassez.[5] The tooth was well formed, except that the amount of enamel was disproportionately large. Parts of its structure are shown in figure 6.

Similar observations were made in the case of other teeth, though minor variations occurred. Some were found to be connected with the surface epithelium, which was of the same type as that seen in the cyst. In a few cases, the amount of squamous epithelium was much greater and showed the development, in addition to the tooth, of subjacent sebaceous glands and hair follicles.

COMMENT

In the interpretation of these observations, certain difficulties that are inherent in all cases of both macroscopic and histologic examination arise.

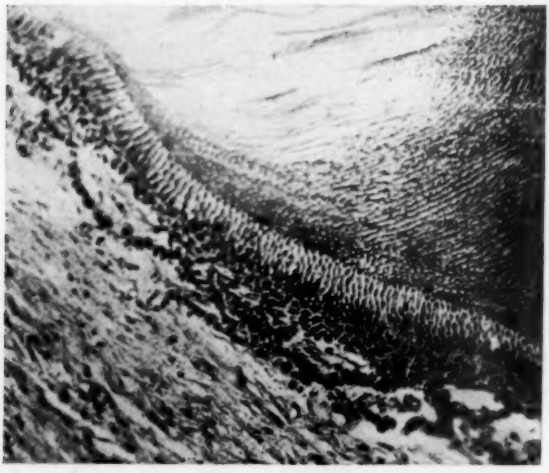

Fig. 6.—Portion of tooth showing ameloblasts and enamel; × 130.

The examination of tissue that has been fixed and mounted—and at present it is not possible to make an adequate examination of tissue prepared by any other method—is necessarily the observation of cells at one definite period of their existence. It is comparable with the examination of a unit section of cinematograph film with an attempt to interpret the preceding and succeeding events from the one picture.

The problem is that of measuring the relationship of the various cells to each other. We have only the relative positions of the cells and their morphology, and what is required in addition is the time

5. Malassez, L. C.: Arch. de physiol. norm. et path., 1885, p. 378; quoted from Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1928; Malassez, L. C., and Galippe, M. L. V.: Les débris epithéliaux paradentaires, Paris, Masson & Cie, 1910.

relationship, so that a proper interpretation of the sequence of events may be established. In this problem is contained that of the direction in which the change in cell structure occurs. Here somewhat arbitrary criteria are used, though continued observations support their use. Thus where relatively undifferentiated cells are present together with differentiated varieties, it is assumed, unless there is definite evidence to the contrary, that the differentiation is proceeding in a manner similar to that observed in the adult body, i. e., from the undifferentiated to the more differentiated type. Also when neighboring cysts possess similar structure, a differentiated portion of the wall of one may reasonably be regarded as a development from the preponderating tissue.

With these difficulties of interpretation in mind, we may now proceed to the interpretation of our observations. As stated previously, that well formed teeth may be present in a region in which there is well formed squamous epithelium is a common discovery. From the considerations discussed, these examples are of little value in helping us to determine the mode of origin of the teeth. The presence of the squamous epithelium at a late stage does not prove that the cellular changes have been identical with those found in other parts of the body. We have a stationary picture from which it is possible to arrive at a knowledge of the changes occurring in the cells only by a correlation of various stages to be found by the study of numerous examples. It is essential to examine early specimens. Such a one is here submitted.

The greater part of the cyst was lined by neuroglial tissue, in some parts with and in other places without a pseudostratified (? ependymal) layer of epithelium. The teratoma frequently is composed principally of nervous tissue.

The additional reasons for assuming that this is the primary form of tissue in the cyst are:

1. It is the most undifferentiated tissue present.
2. Many neighboring cysts have similar walls.
3. The tissue occupies the greater part of the wall of the cyst.

The first significant observation is the development, from the nervous epithelium, of "mucous" glands (fig. 3). Such epithelium would be usually described as "respiratory," but here it is continuous with nervous tissue. It is not necessary to emphasize this feature here, since it has been dealt with by Budde [6] in his discussion of a sacral teratoma. That this epithelium which is producing the glands is of the same type as that lining the greater part of the cyst, owing to some alteration in its form and not to a junction of different kinds of epithelium, is shown

6. Budde, M.: Beitr. z. path. Anat. u. z. allg. Path. **68**:512, 1921.

by the observations that they merge gradually one into the other. Moreover, similar glands have been described as occurring in typical nervous tissue in the adult.

In the depths of the crypt described (corresponding to a dental groove), there is, for a short distance, a small area of squamous epithelium. From the area of squamous epithelium to the region of the tooth, the interpretation is apparently easy, since here we have the growing down from the epithelium of cells of definite form toward the piece of bone tissue, and here differentiation in conjunction with that of some of the connective tissue occurs in a definite manner to form a tooth.

If we use the terminology of the old hypothesis, in this small cyst we have brain tissue becoming converted into, or at least directly continuous with, respiratory tract and this in turn joining a dental groove from which a tooth is formed. Why should brain (ectoderm) and respiratory tract (entoderm) occur in continuity unless it is in the region of the neurenteric canal and if they represent this region, why is there development of teeth?

Even if we should ignore the "mucous" glands or appreciate that they arise from nervous tissue, why should the squamous epithelium of the dental groove develop from nervous tissue? Both are ectodermal, but such development suggests the differentiation of cells under the control of some unusual stimulus, rather than an attempt at formation of an embryo.

The reason for the development cannot be supplied in the present state of our knowledge. We know that a potent factor in the differentiation of tissues is their interrelationship with each other. The development of the lens from the epithelium lying above the optic vesicle, even when this is transplanted to the posterior part of the body, is a well known example. The formation of muscle in the tissue lying beneath epithelium of certain types is well substantiated. The presence of the bone in the neighborhood of the cyst in the present case may possibly be a factor in the causation of the epithelial downgrowth. That it is not the only one is shown by the presence of bone in other parts without formation of teeth. On the other hand, the development of the bone may be secondary to the epithelial development. The simultaneous development of teeth from two separate cysts in relationship to one bony mass, in one case, suggests indeed the former sequence of development as the more probable.

The important feature in this case, however, is the development of the tooth from "nervous" tissue. In the sections, the tooth appears to arise from a very small area of squamous epithelium; i. e., there is a small area of squamous epithelium at the site of origin of the dental epithelium. As pointed out, however, the present occurrence of

squamous epithelium does not necessitate the presence of this tissue at the time of origin of the downgrowth of the tooth. In fact, the small amount of this tissue, as shown by serial sections, suggests that it is a recent occurrence—there is evidence that squamous epithelium in "dermoid" cysts readily replaced other epithelia and developed concomitantly with the growth of the tooth. In any case, the tooth has developed directly or indirectly from nervous tissue.

If the tooth has developed from nervous tissue, still another problem arises. Is the "nervous" tissue really of this type, or is our interpretation inaccurate? We have only the morphology of the tissue to depend on, but this appears to be indisputable. In any case, should it be a modification of connective tissue, a great deal of the evidence for the old hypothesis disappears. The nervous or neuroglial nature of this tissue will be accepted for the present.

There is, therefore, in this example, an interrelationship of cells and tissues which is different from that seen in ordinary development. It seems certain that many of the cells of the body possess capabilities for the formation of tissues of other types which are not usually manifested. The exhibition of these potentialities presupposes that stimuli are governing cells in a manner different from that in which they are governed normally.

Further consideration of observations reveals other difficulties. As stated, teeth are usually said to be present in a "mouth" and other structures e. g., "limbs," to be situated on the surface of the "embryo." Many examples of teratoma, however, are multilocular, e. g., those of Shattock and Pye-Smith.[7] If attention is directed to one loculus, how are the remainder to be explained? Shattock's suggestion of twin and triple teratomas only adds to our hypotheses—and to our difficulties. In the case described, the developing tooth is in relationship to one of many cysts that do not communicate with the surface of the "rudiment." The small cyst cannot thus represent a mouth.

When we turn to general biology for assistance, we find some illuminating observations. The hypothesis of the interdependence of tissue growths is supported by numerous examples. Not only has this been found in ordinary biologic investigation, but the same phenomenon has been demonstrated recently by Huggins,[8] who implanted epithelium from the bladder, ureter and renal pelvis in fibrous areas of the body with subsequent formation of bone in the region of the proliferating epithelium. We have confirmed this experimental result.

Further relevant information is to be obtained from Murray's work[9] on the transplantation of the blastoderm of the chick. He grafted

7. Pye-Smith, P. H.: Tr. Path. Soc., London **37**:499, 1886.
8. Huggins, C. B.: Arch. Surg. **22**:377, 1931.
9. Murray, P. D. F., and Selby, D.: J. Exper. Biol. **7**:404, 1930.

the entire blastoderm and also portions into the same or other eggs. He found that in the case of the entire blastoderm, the degree of histologic differentiation was comparable with that found in normal chicks of an equivalent age, but in the case of partial grafts the degree of differentiation fell short of this. His original paper should be referred to.

In another paper,[10] one observation is of particular importance. Murray noted during the differentiation of this tissue a considerable number of accumulations of cells, more or less resembling the developing organs, but having no counterpart in the normal developing organism, and he referred to these as "pseudo-organs." If we should refer, in our descriptions and discussions of the complex developments in these teratomatous growths, to pseudo-organs—if we must refer to organs at all—we should be much nearer the truth and therefore considerably nearer an understanding of these growths.

In still another paper,[11] Murray gave drawings of his grafts that, in the absence of the knowledge of the grafting, would be accepted as pictures of teratoma. The developing graft forms as a nodule of tissue showing considerable differentiation in various directions occuring in one portion of the wall of a cyst ("vesicle"). Small cavities that he described as "skin vesicles" and "gut" and nodules of cartilage are to be found in the nodule of tissue. Since the grafts were taken from the paravertebral tissue behind formed somites of the chick, it is apparent that complicated structures may result from the development of tissue away from the vertebral region.

How we are to apply such observations to the development of teratoma is still obscure, but it indicates an avenue of research. Budde,[6] in an epoch-making paper, gives reasons for considering that the teratoma is derived, not from an ovum or blastomere, but from a portion of the primitive streak. The suggestions derived from experimental biology indicate that probably it will not be necessary to go back, in the development of the individual, even as far as this for the tumor anlage.

SUMMARY

The early development of teeth in a dermoid cyst is described and illustrated. They are shown to develop, in some cases, from primitive nervous tissue. The development of a tooth from the epithelium of a closed cyst and from tissue which, in the normal body, has no direct relationship to it, indicates that its development is the result of differentiation in tumor tissue which properly is not comparable with the growth of an embryo.

10. Murray, P. D. F., and Huxley, J. S.: Brit. J. Exper. Biol. **3**:9, 1925.

11. Murray, P. D. F.: Australian J. Exper. Biol. & M. Sc. **5**:237, 1928.

EXPERIMENTAL PATHOLOGY OF THE LIVER

II. EFFECT OF CHLOROFORM ON THE NORMAL LIVER AND ON THE RESTORED LIVER FOLLOWING PARTIAL REMOVAL

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The specific action of chloroform on hepatic tissue is well known although the mode of its action is still undetermined. Chloroform induces central necrosis of the lobules of the liver in animals whether administration is by inhalation, mouth or subcutaneous injection. Similar lesions have been demonstrated in man after anesthesia with chloroform.

The remarkable regenerative powers of the liver have been demonstrated by many workers. From two thirds to three fourths of the liver may be diseased or destroyed by toxic substances, and yet it will recover its normal size and weight in a surprisingly short time. Whipple and Sperry [1] showed that lesions in the liver due to chloroform were completely repaired in eleven days when as much as two thirds of the organ was involved. Whipple [2] also showed that puppies during the first three weeks of life are less susceptible to injury by chloroform than mature dogs, and he attributed this to a protective mechanism associated with the blood-forming islands which occur in the sinusoids of the liver in newborn animals.

Since the liver of the very young animal is more resistant to chloroform, I was interested to know whether the lobe of the liver of the white rat that increases so rapidly following partial hepatectomy, displaying embryonic or fetal tendencies, is any more or less resistant to chloroform than the liver of the normal animal.

METHODS OF INVESTIGATION

Only healthy rats, aged from 3 to 6 months and weighing from about 100 to 225 Gm., were used. The central and left lobes, comprising about 65 per cent of the entire liver, were removed according to the method of Higgins and Anderson.[3] Six weeks later, chloroform was administered. Histologic studies were made of the development and the repair of the hepatic lesions following the administration of

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From the Division of Experimental Surgery and Pathology, the Mayo Foundation.

1. Whipple, G. H., and Sperry, J. A.: Bull. Johns Hopkins Hosp. **20**:278, 1909.
2. Whipple, G. H.: J. Exper. Med. **15**:259, 1912.
3. Higgins, G. M., and Anderson, R. M.: Arch. Path. **12**:186, 1931.

choloroform in both normal and recently restored hepatic tissue. Only chloroform, U. S. P., "for anesthesia," was used.

The administration of chloroform by inhalation resulted in such slight and in most cases negligible lesions of the liver that the method was abandoned. On the other hand, the introduction of chloroform and liquid petrolatum in equal parts into the stomach produced such severe injury that the rats died in a few hours. Hemorrhagic lesions of the peritoneum, pleura, intestines, stomach and kidneys occurred. Bloody fluid was found in the pleural cavities and in the peritoneum. Gross hematuria was noted within a few minutes. A single subcutaneous injection of this mixture, however, proved to be a most efficient method for producing hepatic lesions. Central necrosis of the lobules of the liver occurred in the majority of rats so treated, and a dose that, if given by stomach, killed the rats in a few hours could in this way be safely administered.

Effect of Chloroform on the Normal Liver and on the Restored Liver After Partial Removal

Livers of Normal Rats				Restored Livers			
Weight of Rat, Gm.	Dose of Equal Parts of Chloroform and Oil, Cc.	Time Killed, Hours After Injection	Hepatic Injury*	Weight of Rat, Gm.	Dose of Equal Parts of Chloroform and Oil, Cc.	Time Killed, Hours After Injection	Hepatic Injury*
98.5	0.25	24	None	214	0.75	24	Slight
204	0.75	24	26.4	207	0.88	24	Slight
215	0.88	24	Slight	165	0.75	24	None
157.2	0.5	24	30.7	205	0.5	48	Slight
119	0.25	48	65.0	120	0.5	48	Slight
183	0.5	48	31.0	205	0.75	48	12.2
102	0.5	48	42.0	96	0.5	48	9.3
106	0.5	48	44.0	160	0.5	48	25.0
144	0.5	48	38.0	188	0.75	48	Slight
161	0.75	48	34.5	182	0.75	48	16.4
95	0.5	72	None	223	0.88	48	Slight
125	0.5	72	10.4	153	0.75	48	None
145	0.5	72	None	119	0.5	72	48.4
156	0.5	72	56.4	153	0.5	72	Slight
97	0.25	72	24.3	158	0.5	72	11.3
140	0.5	72	10.4	204	0.75	96	8.3
140	0.5	96	20.8	206	0.88	96	None
103	0.5	96	20.8	206	0.88	96	None
201	0.88	96	Slight	197	0.88	96	None
				124	0.75	96	None

* Figures indicate percentage of hepatic tissue that was necrotic.

A method of subcutaneous administration was devised that prevented leakage and insured accurate dosage. The needle was inserted through the abdominal skin into the muscular layer, then directed forward through the muscle for a distance of 1 cm. and then superficially into the subcutaneous tissues, where the mixture of chloroform and oil was deposited. The needle was withdrawn, and the pressure of the mixture obliterated the passageway of the needle through the muscle and thus prevented loss of the chloroform.

The amounts of the mixture of equal parts of chloroform and liquid petrolatum used were as follows: for rats weighing less than 100 Gm., 0.25 cc.; for rats weighing from 100 to 150 Gm., 0.5 cc.; for rats weighing from 150 to 200 Gm., 0.75 cc., and for rats weighing more than 200 Gm., 0.88 cc.

Sixty-eight rats not operated on were given subcutaneous injections. Of these, twenty-two (33.9 per cent) died within the first four days. All had definite lesions in the liver.

Fifty-four rats in which partial hepatectomy had been done six weeks before were given similar injections. Ten of these (19 per cent) died within the first five days, and two died on the fifth day.

The surviving rats were killed at daily intervals after injection up to the twelfth day. Some of the rats killed the first three or four days might have died if they had been allowed to survive, so the percentages stated are not absolute figures for mortality; nevertheless they indicate that more of the normal rats died following the injection of chloroform than rats in which partial hepatectomy had been done.

Histologic studies were carried out on the livers of all the rats that were killed and of a few of those that died in which autolysis was not present. The following staining mediums were used: hematoxylin and eosin; sudan III; Mallory's connective tissue stain; van Gieson's connective tissue stain; del Rio Hortega's gold chloride ammoniacal silver carbonate, and del Rio Hortega's ammoniacal silver carbonate cold for mitochondria.

OBSERVATIONS

The central necrosis that occurred in the lobule of the liver after the subcutaneous injection of chloroform was more or less alike in both the normal and the newly restored liver except in extent. Necrosis following comparable injections was always far less in the restored liver than in the normal liver, but the description that follows is applicable to either of the two types of livers.

Six hours after an injection of chloroform there was slight microscopic evidence of chloroform injury, although grossly the liver appeared entirely normal. These slight changes involved distention of the sinusoids and occasionally disruption of the endothelium of the central veins, and there was a definite increase of fat in the paler staining central areas. Definite necrosis about the central veins occurred eighteen hours after the injection. The sinusoids were obliterated in these areas of necrosis, owing essentially to the swelling of the cells, and the endothelium of the central veins invariably showed disruption. A marked deposit of fat occurred in the necrotic areas as well as in the periphery of these zones where there was no definite necrosis.

Twenty-four hours after the injection, necrosis was marked. The cells were swollen, and the nuclei were pyknotic, small and often absent. Many cells had fragmented, and wandering mononuclear cells had invaded the necrotic zones to remove dead cells and other débris. Sudan III stains showed marked fatty degeneration, and necrotic cells were more hyaline and did not stain with any of the staining technics employed. At the periphery of the lesion where fatty changes were more marked, the cells were extensively vacuolated.

Marked central necrosis occurred forty-eight hours after the injection (figs. 1 and 2). The hepatic reticulum, however, still persisted and was apparently unaffected by the chloroform. Mitotic figures were abundant at forty-eight hours after the injection, and as many as ten or twelve figures were often identified in one field. They were usually situated just peripheral to the areas of fatty change.

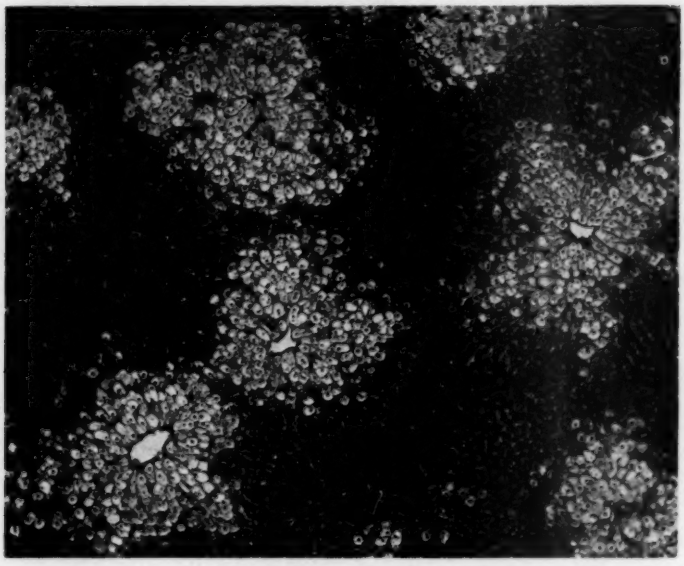

Fig. 1.—Liver of a normal rat forty-eight hours after injection of chloroform (del Rio Hortega technic, cold method; × 60).

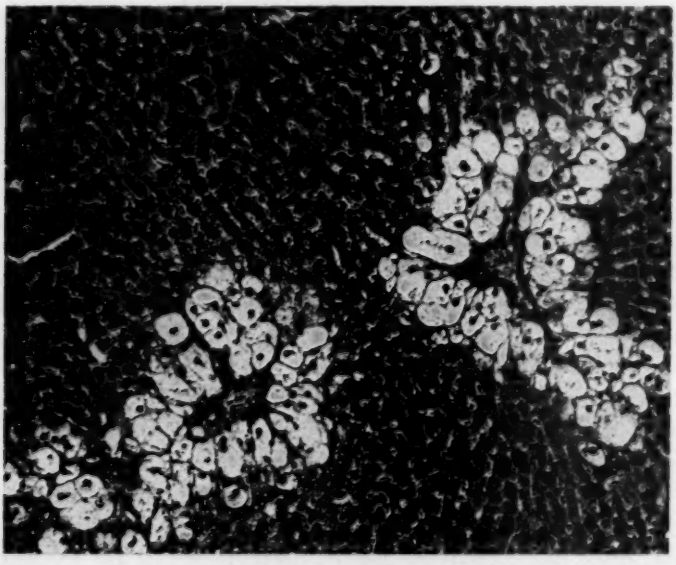

Fig. 2.—Restored liver of a rat forty-eight hours after injection of chloroform (× 125).

As soon as necrosis had begun, phagocytic cells entered the necrotic areas. They were mainly wandering monocytic cells, but there were some polymorphonuclear leukocytes. This cellular response was first observed twenty-four hours after the injection of chloroform, and it became marked on the second day, attaining its height on the third day (fig. 3). Giant cells, which have been reported by some observers of chloroform poisoning in other laboratory animals, were not seen in the rat. When the removal of necrotic cells and material from the central areas continued, mitosis progressed vigorously, and new cells pushed into the areas that were formerly necrotic. The portal spaces were never

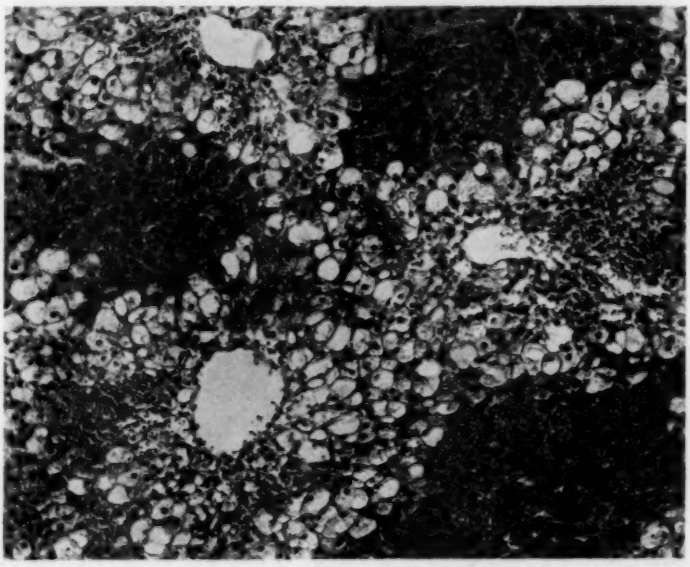

Fig. 3.—Liver of a normal rat seventy-two hours after injection of chloroform. Repair is in progress; infiltration by phagocytic cells may be observed (× 100).

involved, and cytologic changes were not observed in the portal spaces or in the cells surrounding them.

On the fourth day, the extent of the lesion was appreciably less. Phagocytic cells were still present, but the greater part of the necrotic material had been removed, and the necrotic areas were filled to a great extent by new cells, which had crowded in from the adjacent normal areas. Reticulum may play a part in the repair of the lesion, for the ammoniacal silver carbonate stains showed that these fibers persisted during the third and fourth days when the greater part of the necrotic material had been removed. Even the finest fibrils were uninjured, and it is probable that the reticulum proved a framework along which the new hepatic cells migrated into the formerly injured areas.

On the fifth day, the repair was complete, and the necrotic materials had been removed (fig. 4). The endothelium of the central veins was often incomplete, and the sinusoids were quite irregular; but new hepatic cells occupied the central spaces that had formerly contained the necrotic material.

The literature on the form and distribution of mitochondria in the animal cells is enormous, and no attempt will be made to review it here except to quote Cowdry's definition.[4] Cowdry assembled and reviewed more than five hundred papers on mitochondria and defined mitochondria as follows: "Substances which occur in the form of granules, rods

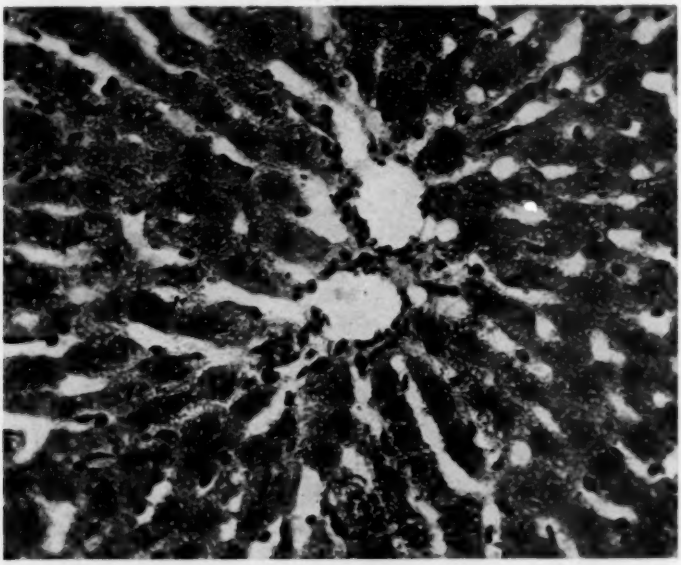

Fig. 4.—Liver of a rat on the fifth day after injection of chloroform. Repair of the lesion is essentially complete (× 250).

and filaments in almost all living cells, which react positively to Janus green B and which by their staining reactions and solubilities resemble phospholipins and to a lesser extent albumins." Cowdry regarded them as the most delicate indicators of cellular injury. The del Rio Hortega ammoniacal silver carbonate cold method for mitochondria was selected as the most reliable of the several methods that were employed.

Normally the mitochondria of the hepatic cell are fine rods and fine granules scattered rather uniformly throughout the cell, but immediately following the absorption of chloroform, they change from rods and small granules to coarse spherical granules (figs. 5 and 6). This demon-

4. Cowdry, E. V.: General Cytology, Chicago, University of Chicago Press, 1924, p. 313.

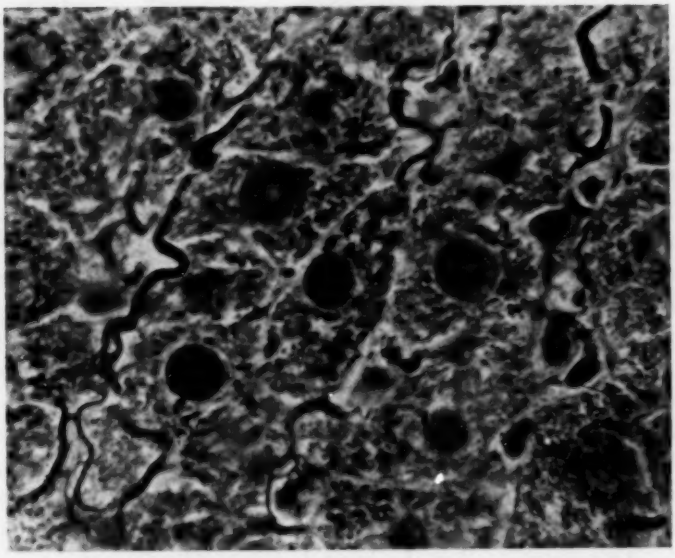

Fig. 5.—Mitochondria in the liver of a rat (del Rio Hortega technic, cold method; × 1,250).

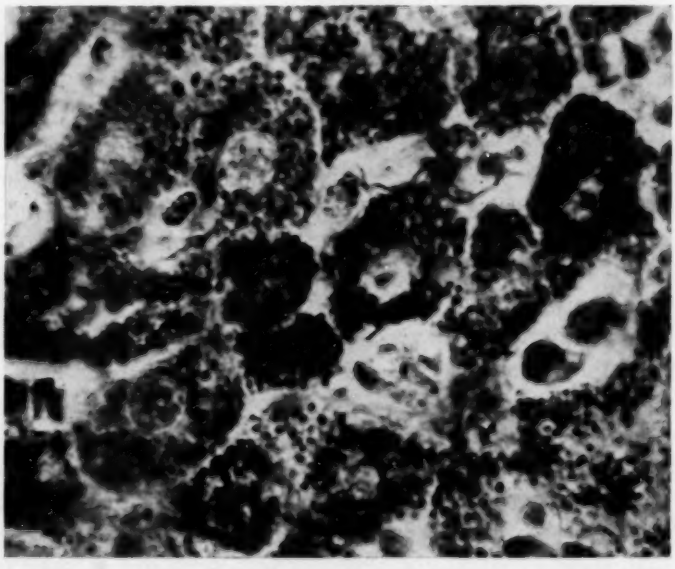

Fig. 6.—Mitochondria in the liver of a rat (del Rio Hortega technic, cold method; × 1,250).

strates their delicate and immediate reaction to chloroform. This change in shape of the mitochondria was not limited to the central areas of necrosis, but was uniform throughout the lobules. Twenty-four hours after the administration of chloroform, however, the mitochondria were normal again, appearing as fine rods and fine granules, and could not be distinguished from the mitochondria of a liver that had not been exposed to the action of chloroform.

The extent of the lesion in both the normal and the restored livers was determined by the use of a projectoscope of the Eddinger type. Six representative fields from the microscopic sections obtained from each rat in which the hepatic lesion was definite enough for accurate determination were projected on uniform white cardboard. The outlines of the necrotic areas were traced and cut out, and their weight was compared with the weight of the entire tracing. This gave an approximate estimate of the percentage of the lobule that had sustained necrosis. The data, which have been condensed into table 1, rather definitely show that the extent of the lesion was far greater in the normal rats not operated on than in those possessing a newly restored organ following partial removal. This suggests that the liver that is restored so rapidly following operation may possess such protective agencies as to make it comparable to the hepatic organ in the new-born rat and thus less susceptible to chloroform.

COMMENT

Chloroform induced a specific destructive lesion in hepatic tissue and also profound lesions in the heart and kidneys. Large doses caused death before a demonstrable hepatic lesion had time to occur. Mitochondrial stains indicated that the chloroform affected the hepatic cell within a few minutes of the time of its administration. The lesion produced in the liver by chloroform was a central necrosis associated with fatty changes. In some cases, as much as from two thirds to three fourths of the liver was destroyed by chloroform, and yet the animals survived and entirely recovered.

The extent of the lesion varied with the method of administration. When chloroform was administered by inhalation to either normal rats or those with recently restored liver after partial hepatectomy, slight, negligible lesions occurred. This probably illustrates the protective action of a well balanced diet. When chloroform was administered by stomach, such severe lesions occurred that the rats did not survive through the day of injection, in spite of the fact that the doses administered were no greater than those given by the subcutaneous method. Marked hemorrhagic lesions were present in the pleura, peritoneum, stomach, intestines, liver, kidneys and spleen. The coagulation factors of the blood were altered, and the peritoneum and pleural cavities contained large amounts of hemorrhagic fluid. Hematuria also occurred.

The subcutaneous injection of chloroform and sterile liquid petrolatum in equal amounts proved excellent. In some cases, the lesion was slight, involving only a few cells about the central vein and disruption of the endothelium of the vein with widening of the sinusoids due to congestion; in other cases, it was so severe as to involve as much as two thirds of the lobule of the liver. The extent of the lesion was not always proportional to the dose of chloroform administered; a stated dose given to a small rat did not in some cases produce as severe a lesion as the same dose given to a larger rat. Other factors also entered.

Evidence of necrosis was sometimes found at eighteen hours, but it was never extensive, whereas at twenty-four hours the lesion was always well defined. The lesion was most marked, however, on the second and third days after the administration of chloroform. Repair of or regeneration of the liver, as indicated by mitosis, occurred as early as twenty-four hours after the injection of chloroform, but it, too, was marked at forty-eight and seventy-two hours. Mitotic figures were most abundant in normal hepatic cells in the periphery of the lobule between the area of fatty changes and the portal spaces.

Repair was aided by the wandering phagocytic cells, which largely removed the necrotic tissue by the fourth day. At the fifth day, repair, it may be said, was 95 per cent complete. It was entirely complete by the sixth or the seventh day, when the endothelium of the central veins had been reestablished, and fatty changes had disappeared. Van Gieson's stain and Mallory's connective tissue stain indicated no activity of fibroblastic elements in the repair of the lesion produced by chloroform.

Repair of the lesion produced by chloroform took place in recently restored hepatic tissue exactly as in normal hepatic tissue. Since the lesion was less extensive in recently restored hepatic tissue, complete repair was more rapid in the rats that had been partially hepatectomized than in the normal rats. This probably was due to the lesser extent of the lesion and not to any greater power of repair on the part of the recently restored hepatic tissue.

A comparison of the two groups of rats from the standpoint of the extent of the lesions induced and the mortality encountered leads one to conclude that recently restored hepatic tissue is either more resistant or perhaps less susceptible to the effects of chloroform than normal hepatic tissue.

SUMMARY

Normal rats and those with recently restored hepatic tissue after partial hepatectomy, when maintained on standard rations, were very resistant to the action of chloroform administered by the inhalation method, but were rapidly killed by chloroform administered by stomach tube.

Injection of chloroform and sterile liquid petrolatum, in equal parts, into the subcutaneous tissues of the abdominal wall proved an easy and accurate method of administration, although the lesion produced was not invariably proportional to the dose. In some cases, a small rat did not have as extensive a lesion as a larger rat receiving the same dose.

Central necrosis occurred on the first day, but was much more marked on the second and third days after the injection. In the normal rat, repair was almost complete at the fifth day. There was no scar tissue. The lesion was far less extensive in the newly restored liver following partial removal than in the normal liver, and repair was more rapidly complete in the animals operated on than in those with intact livers. This may have been due to the lesion being less extensive and not to greater reparative power.

When the extent of necrosis was determined by graphic methods, normal rats showed a much higher percentage of necrosis than rats with a recently restored organ.

A larger percentage of normal rats died (33.9 per cent) after subcutaneous injection of chloroform than rats with recently restored hepatic tissue (19.2 per cent). These data lead one to conclude that recently restored hepatic tissue is more resistant or less susceptible to chloroform than the liver of normal rats.

NORMAL FAT CONTENT OF THE KUPFFER CELLS

HISTOLOGIC STUDY

VICTOR LEVINE, M.D.

CHICAGO

The so-called Kupffer cells of the liver were first described in 1869 by Ponfick,[1] who discovered them as stained elements attached to the walls of the hepatic sinusoids, by injecting mercuric sulphide and ultramarine blue. From that day to this the phagocytic abilities of the cells have never been in doubt. There has, however, been considerable dispute as to the exact nature and origin of the cells. Von Kupffer [2] in his first work in 1876 believed the cells to be perivascular, not endothelial. By the time of his last paper, in 1899,[3] he had completely changed his opinion and considered them to be purely endothelial. Since then their endothelial, or reticulo-endothelial, nature has not been in serious dispute.

There is still disagreement as to whether or not all the cells lining the sinusoids, the endothelial cells, are in fact Kupffer cells which can assume phagocytic properties. Von Kupffer's [3] final opinion was that they were the only endothelial cells of the capillaries. Schilling [4] believed that it was impossible to differentiate Kupffer cells and endothelial cells histologically, and that all actively phagocytosing Kupffer cells develop from preexisting endothelial lining. Nathan,[5] studying human embryos, concluded that the evolution of the hepatic endothelium was the same as that of all reticular apparatus, and that the Kupffer cells were the only vascular endothelium. Maximow [6] thought that practically all the cytologic elements of the capillary wall might assume the properties of Kupffer cells. Higgins and Murphy [7] and Higgins and Mann,[8] using injections of graphite suspensions, came to the conclusion that not all the endothelial elements are capable of phagocytosis. On the

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1. Ponfick, E.: Arch. f. path. Anat. u. Physiol. **48**:1, 1869.
2. von Kupffer, C.: Arch. f. mikr. Anat. **12**:353, 1876.
3. von Kupffer, C.: Arch. f. mikr. Anat. **54**:254, 1899.
4. Schilling, V.: Centralbl. f. allg. Path. u. path. Anat. **19**:577, 1908.
5. Nathan, M.: La cellule de Kupffer, Thèse de Paris, no. 118, 1908.
6. Maximow, A. A.: The Macrophages or Histiocytes, in Cowdry, E. V.: Special Cytology, New York, Paul B. Hoeber, Inc., 1928, pp. 425-484.
7. Higgins, G. M., and Murphy, G. T.: Anat. Rec. **60**:15, 1928.
8. Higgins, G. M., and Mann, F. C.: S. Clin. North America **9**:63, 1929.

basis of experimental work, Pfuhl [9] divided the Kupffer cells into the seizing, projecting type, and the flat, digesting type. As the result of studies on human material, Schilling [4] made the statement that the flat endothelial cells were resting cells which became much swollen during phagocytic activity and later, when completely saturated with the engulfed particles, assumed an intermediate, oval form.

Nathan [5] divided the functions of the Kupffer cells into three groups: phagocytosis of large elements such as blood cells or cell fragments, phagocytosis of bacteria and phagocytosis of granules, including fat granules. Von Platen [10] in 1878 was the first to report phagocytosis of fat. Soon afterward various workers found carmine, erythrocytes, leukocytes, colloidal silver and other substances as inclusions within Kupffer cells. Schilling [4] was the first to describe phagocytosis of bacteria in detail. More recently Aschoff and Kiyono,[11] on the basis of intravital staining with lithium carmine, definitely considered Kupffer cells as a part of the reticulo-endothelial system.

Gilbert and Carnot [12] made experiments with the injection of fat and found the fat at first in the Kupffer cells and only after some hours in the hepatic cells. They inferred that the Kupffer cells passed the fat to the hepatic cells. Nathan [5] injected stearic acid and linseed oil and found inclusions of fat particles in the Kupffer cells. Saxl and Donath [13] injected a fine fat emulsion and after watching its disappearance from the blood reported their results as a basis for a functional test of the reticulo-endothelial system. That it is not such a test was shown in the experiments of Jaffé and Berman,[14] who, working with the same emulsion, found that the fat was taken up almost immediately by the Kupffer cells of the liver and not to any marked extent by the reticulo-endothelium elsewhere. These investigators also stated that the fat droplets were passed quickly to the hepatic cells from the Kupffer cells, especially in the peripheral acinar zones. Thus they felt that the injection of this particular emulsion tests chiefly the hepatic function, particularly the relations between hepatic and Kupffer cells, and that its use is in no sense a test of function for reticulo-endothelium in general. In this regard it may also be mentioned that Sacks [15] injected

9. Pfuhl, W.: Ztschr. f. Anat. u. Entwcklgsgesch. **81**:90, 1926.

10. von Platen, O.: Virchows Arch. f. path. Anat. **74**:268, 1878.

11. Aschoff, L., and Kiyono, K.: Verhandl. d. deutsch. path. Gesellsch. **16**:107, 1913. Kiyono, K.: Die vitale Karminspeicherung; ein Beitrag zur Lehre von der vitalen Färbung mit besonderer Berücksichtigung der Zelldifferenzierungen im entzündeten Gewebe, Jena, Gustav Fischer, 1914. Ashchoff, L.: Lectures on Pathology, New York, Paul B. Hoeber, Inc., 1924, p. 7.

12. Gilbert, A., and Carnot, P., quoted by Nathan.[5]

13. Saxl, P., and Donath, F.: Wien. klin. Wchnschr. **38**:66, 1925.

14. Jaffé, R. H., and Berman, S. L.: Arch. Path. **5**:1020, 1928.

15. Sacks, B., quoted by Aschoff: Lectures on Pathology, New York, Paul B. Hoeber, Inc., 1924, p. 94.

a fine emulsion of cholesterol, and found that it was deposited in the cells of the reticulo-endothelium.

Jornier [16] made experiments feeding fat and observed fat in both the hepatic and the Kupffer cells in no constant proportion, although he did find it especially marked in the Kupffer cells of the peripheral acinar zones. He found that the fat first increased in amount in the hepatic cells and then began to decrease from the eleventh hour after feeding, at which time the amount of fat in the Kupffer cells started increasing. This would seem to indicate that the fat passed in the reverse direction, from the hepatic to the Kupffer cells. More work on this type of experiment would seem to be necessary to determine the correctness of these results, and to explain the great difference in the findings of the two types of experimentation.

Little has been written about the presence of fat in the Kupffer cells in pathologic conditions of human beings. Schilling [4] mentioned a case of lipemia and pernicious anemia in which both fat and iron were found in the Kupffer cells, but only iron in the hepatic cells. He concluded that the lipemia was of short duration, and that the fat was actively removed from the blood by phagocytosis. In his experimental work, however, he found fat in varying proportions in hepatic and Kupffer cells, and believed that the same poison that caused fatty changes in the hepatic cells also caused the Kupffer cells to take up fat. Nathan [5] mentioned the findings in many different pathologic conditions. In connection with only one case, however, does he speak of fat in the Kupffer cells. This was a case of eclampsia in which both mother and child had marked swelling of the Kupffer cells, which were stuffed with fat.

Fischer [17] studied the localization of fat in the livers of 150 patients who died from natural causes. The livers of new-born infants and livers with gross destructive lesions, such as abscesses or cirrhosis, were excluded. None of the 150 livers was free from fat. He found no correlation between the state of bodily nutrition and the amount of fat in the liver. He also concluded that the length of time between death and postmortem examination had no influence on the amount of fat in the liver. In 3 of these 150 cases, fat was found solely in the Kupffer cells. In 43 cases, there was no fat in the Kupffer cells; in 59 cases, they were all filled with fat. In a similar series of 150 human livers, Helly [18] found fat in the Kupffer cells of 40 per cent. Neither Fischer nor Helly could discover any relation between the amount of fat in the Kupffer cells and the amount or localization of fat in the hepatic cells. Fischer also stated that there was no relation between the

16. Jornier, quoted by Nathan.[5]

17. Fischer, W.: Virchows Arch. f. path. Anat. **208**:1, 1912.

18. Helly, K.: Beitr. z. path. Anat. u. z. allg. Path. **51**:462, 1911.

cause of death and the amount of fat in the Kupffer cells, and considered that the fat in the Kupffer cells was not characteristic of an infectious process, since it was completely absent in several patients dying of infections, such as pneumonia or peritonitis. In livers with definite stasis, he found fat more frequently present in the Kupffer cells than in the hepatic cells. He granted the usual presence of fat in the Kupffer cells of the gravid liver, and he was in accordance with Rössle [19] on findings in the diabetic liver.

Rössle [19] observed that in the diabetic liver the Kupffer cells were almost always stuffed with fat. In his opinion, this, with increased pericapillary connective tissue, made the picture diagnostic of diabetes. Helly,[18] while agreeing as to the finding of fat in the Kupffer cells, did not consider the presence of fat as pathognomonic.

Thus no definite conclusions have been reached as to the causes of the appearance of fat in the Kupffer cells. All that can be said is that most of the workers have considered its presence as pathologic (Schilling, Nathan, Rössle).

No reference has been found to a study of the normal lipoid content of the Kupffer cells or to the possibility of fat being normally present in the Kupffer cells. Fischer [17] left one to infer that fat may have been present in the hepatic cells before the development of the disease that caused the patient's death, but he made no direct mention of this possibility. Helly [18] made the statement that, while pneumonia or septic processes (as well as diabetes) seem to cause the appearance of fat in the Kupffer cells, fat is also found there in infection-free persons. He did not, however, mention the possibility that the fat in the Kupffer cells of the last-mentioned group could be a normal finding. Aschoff,[15] in mentioning Sack's work, stated that injected cholesterol localizes to the reticulo-endothelial system, and that this location is different from that in which fat is normally found. Kawamura [20] described the presence of doubly refracting lipoids in the Kupffer cells under different pathologic conditions. It has also been shown by Anitschkow [21] and many other investigators that in the experimental cholesteatosis produced by feeding, the Kupffer cells are always filled with cholesterol and cholesterol esters.

In order to determine the normal fat and lipoid content of the Kupffer cell it was found advisable to examine the liver in persons who had met sudden, violent deaths. Forty-three livers were examined.

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19. Rössle, R.: Verhandl. d. deutsch. Path. Gesellsch. **10**:334, 1907.

20. Kawamura, R.: Neue Beiträge sur Morphologie und Physiologie der Cholesterinsteatose, Jena, Gustav Fischer, 1927.

21. Anitschkow, N.: Deutsche med. Wchnschr. **39**:741, 1913; Med. Klin. **10**: 465, 1914.

METHODS

On formaldehyde-fixed material the following stains were used: sudan III and hematoxylin, nile blue sulphate, Fischler's stain and Lorrain Smith-Dietrich stain. Unstained frozen sections were also examined for doubly refracting lipoid bodies. Pieces of tissue fixed in Ciaccio solution were stained by that method.

In interpreting the results, one must bear in mind that a certain staining reaction is not proof of the presence of a substance in pure state. Since fatty and lipoid substances are generally present as mixtures, the staining properties are due only to the predominance of one type (as neutral fat or fatty acid, for example). Chemical analysis reveals more lipoid material than can be demonstrated by histochemical methods. Thus the procedure used here can in no sense be taken as absolutely determining the type and the amount of each of the various lipoid substances, but only as an indication of the predominating kind present in the various cells.

RESULTS

The table shows the results obtained in the 27 livers examined by all the staining methods. For completeness' sake, the findings for hepatic cells are listed as well as those for Kupffer cells.

In every liver, fat was found in varying amounts in both the hepatic and the Kupffer cells with the sudan III stain. When the Kupffer cells contained much fat and the hepatic cells little, the sudan III stain was almost specific for the Kupffer cells, which, even under low power magnification, stood out as orange-yellow dots. In only 3 cases was the amount of sudanophil material in the Kupffer cells tabulated as low as a trace. In 2 of these cases (22 and 24), brown granules were found in the hepatic cells, which leads one to assume that these livers may not have been completely normal at the time of death. The 16 additional livers not listed in this table were stained only by the sudan III and hematoxylin method. Fourteen showed results similar to those listed, while in 2 the Kupffer cells contained no fat. The latter showed early periportal cirrhosis and can be discarded from the group as abnormal.

On careful examination of the Kupffer cells, the picture closely fitted Fischer's description [17] in which he said that the fat droplets were smaller than the nucleus and usually were arranged in graded rows with the largest droplets next to the central nucleus and the smallest droplets farthest from the nucleus, thus giving the cells a pointed appearance. This is illustrated in the figure. The amount of fat in the hepatic cells varied considerably and bore out Fischer's contention [17] that it had no relation to the state of nutrition, which is listed in the table.

With the nile blue sulphate stain, a variety of results was obtained. In some, all of the granules in the Kupffer cells stained blue; in some,

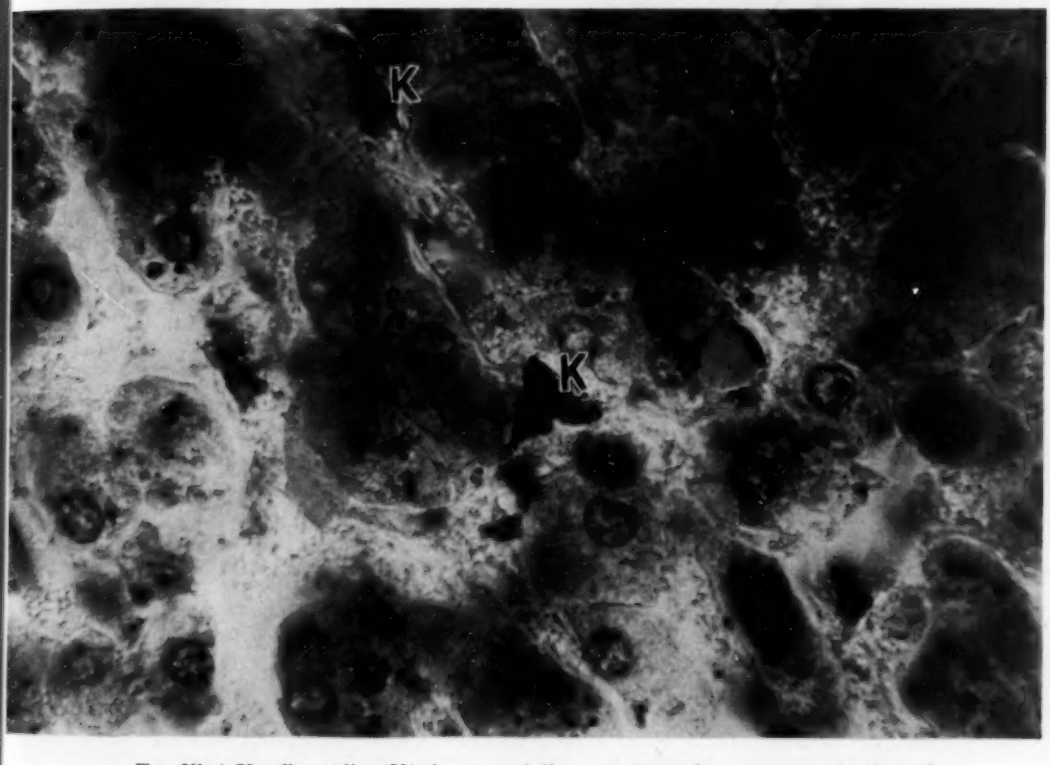
Normal Fat Content of Kupffer Cells as Shown in 27 Livers

Case	Race	Sex	Age	Cause of Death	Time Between Injury and Death	State of Nutrition	Lipin Content of Kupffer Cells: Sudan III	Nile Blue Sulphate	Lorrain Smith-Dietrich Stain	Ciaccio Stain	Doubly Refractile Fat	Lipin Content of Hepatic Cells: Sudan III	Nile Blue Sulphate	Lorrain Smith-Dietrich Stain	Ciaccio Stain	Doubly Refractile Fat
1	W	M	50	Fight and stab wounds	Few min.	Good	+++	Blue	+	0	0	++++	Pink	Trace	0	+++
2	W	M	65	Fell from third floor	15 min.	Good	++	Blue	Trace	0	0	+	Pink	Trace	Trace	++
3	W	M	19	Automobile accident	3 hr.	Good	+	Blue	Trace	0	0	+	Pink	0	Trace	+++
4	W	M	40	Gunshot in head	Inst. death	Good	++	Blue	++	0	0	+++	Pink and purple	+	Faint trace	+++
5	Mex.	M	31	Gunshot in abdomen	3 hr.	Good	+	Blue	0	0	0	Trace	Pink	Trace	Trace	+++
6	C	M	a35*	Stab wound in left axilla	2 hr.	Fair	++	Blue	0	0	0	+	Purple	0	Trace	+++
7	W	M	a65	Beaten about head	Few min.	Good	++	Blue	Trace	0	0	++++	Pink	++	0	+++
8	W	M	54	Gunshot in chest	Inst. death	Good	+++	Blue	+	0	0	++	Blue	+	+	++
9	W	M	a30	Gunshot in head	Inst. death	Good	+++	Blue	0	0	0	Trace	Pink	0	Trace	++
10	W	F	20	Stab wound in heart	Inst. death	Good	+++	Pink and blue	0	0	0	+	Pink	0	+	+++
11	W	M	30	Skull fracture, hit over head	Few hr.	Good	++	Pink	0	0	0	Trace	Blue	+	+	+
12	W	M	a30	Skull fracture	Few min.	Good	++	Pink and blue	0	0	0	+	Purple	+	+	++
13	W	M	72	Automobile accident, ruptured liver, perforated lung	About 2 hr.	Fair	+++	Pink	Trace	Trace	0	+	Pink	+	Trace	++
14	W	M	30	Gunshot in head and heart	Inst. death	Good	+	Blue	0	0	0	+	Blue	Trace	+	+
15	W	M	a28	Gunshot in head	Inst. death	Good	Trace	.....	0	0	0	+	.....	+	+	++
16	W	M	a30	Gunshot in heart and lungs	Inst. death	Good	+	Pink and blue	0	0	0	+	Purple	Trace	Trace	+
17	W	M	a35	Gunshot in lungs	Inst. death	Fair	+++	Blue	0	..	0	+	Blue	Trace	.....	+++
18	C	M	44	Stab wound in heart	Inst. death	Good	++	Pink	0	0	0	+++	Pink	+	Faint trace	+++
19	W	M	a35	Gunshot in heart and lungs	Inst. death	Not good	+	Pink and blue	Trace	Faint trace	0	+	Pink	Trace	Trace	+
20	W	M	a35	Beaten about head	Sudden death	Obese	++	Pink	Trace	Trace	0	+++	Pink	+	Faint trace	++
21	W	M	a35	Gunshot in heart and chest	Inst. death	Good	+	Pink	Trace	Trace	0	+++	Pink and purple	+	0	+++
22	C	M	a35	Gunshot in heart and lungs	Inst. death	Good	Trace	Blue	Faint trace	Trace	0	Trace	Purple	Trace	Trace	+
23	C	M	a25	Gunshot in heart and chest	Inst. death	Good	+	Blue	Trace	Faint trace	0	Trace	Blue	Faint trace	+	++
24	C	M	a50	Gunshot in lungs and aorta	Inst. death	Good	Faint trace	Blue	0	Faint trace	0	Trace	Blue	Faint trace	+	+
25	W	M	40	Gunshot in abdomen	3 hr.	Good	+++	Pink and blue	Trace	0	0	+	Pink, purple and blue	Faint trace	Trace	+
26	W	M	50	Gunshot in left arm with hemorrhage	About 1 hr.	Good	++	Pink	0	0	0	+	Pink	Trace	Trace	++
27	W	F	20	Gunshot in chest	About 30 min.	Good	+	Pink and blue	Faint trace	0	0	++	Pink and purple	Trace	0	Trace

The Fischler stain was also used, but revealed no fat droplets in either type of cell.

* a = about.

pink, and in some, a mixture of blue and pink. The blue-staining granules predominated, however, and this was thought to be due to their smaller size. In the hepatic cells, as a general rule, the large fat droplets stained pink, the medium sized ones purple and the small ones blue. While a pink stain indicates neutral fat and a blue one fatty acid, the surrounding membrane of a fat droplet often contains a thin film of fatty acid and lipoid material, which would stain blue. This probably explains the blue staining of the majority of the smaller droplets.



Fat-filled Kupffer cells (K) in normal liver; reduced from a magnification of × 1,200; formaldehyde fixation; frozen section; sudan III and hematoxylin stain.

With the Fischler stain for fatty acids, no droplets were found in either Kupffer or hepatic cells that took the stain; this clarifies the findings with the nile blue sulphate stain and practically proves that the fat is mainly neutral.

With the Lorrain Smith-Dietrich stain for lipoids, some of the Kupffer cells showed lipoid granules and some did not. No correlation seemed to exist between the amount of sudanophil material in the Kupffer cells and the presence of lipoid granules demonstrable by this method. In most cases, the hepatic cells were positive with this stain,

owing in large part to the fact that the envelop of most of the large fat droplets could be seen to contain lipoids stainable by this method.

With the Ciaccio stain for lipoids, a variety of results was also obtained. Here again there appeared to be no relationship between the findings with the usual sudan III stain and those obtained with this special sudan stain. Most of the hepatic cells had granules that took this stain, while the majority of the Kupffer cells did not. The amount of lipoid found, by this stain, in the Kupffer cells was only very slight.

The examination for doubly refractile bodies disclosed none in the Kupffer cells and a varying amount always present in the hepatic cells. Double refraction of lipoid material is indicative of the presence of considerable amounts of cholesterol and cholesterol esters in the lipoid mixtures. Since Anitschkow [21] has shown that Kupffer cells store cholesterol from the blood stream, the finding probably indicates that under normal conditions the cholesterol is passed to the hepatic cells too rapidly to be demonstrated in the Kupffer cells by the method used here.

SUMMARY

Although Kupffer cells have been described since 1869 and definitely identified as endothelial elements since 1899, scant attention has been given to their fat content under pathologic conditions, and the question of a normal fat content has been neglected.

A study of the histologically demonstrable fat and lipoid substances of both hepatic and Kupffer cells was made on the livers of 27 persons dying a sudden, violent death. The fat content alone was studied in an additional 16 similar cases. In all but 2 of the 43 cases fat was found in the Kupffer cells. In the exceptions, early cirrhosis of the liver was present. In each case, fat was also found in the hepatic cells.

Special stains demonstrated that the sudanophil material in both hepatic and Kupffer cells was mainly neutral in type, and that lipoid substances were at times present in both kinds of cells. Doubly refracting lipoid substances were found in the hepatic cells, but not in the Kupffer cells.

Thus one can conclude that neutral fat is normally found in the Kupffer cells of the liver.

OCCURRENCE OF A CALCAREOUS ARTERIAL LESION IN GOITER

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Curtis [1] and Curtis and Delaney [2] have drawn attention to the occurrence of calcification of the thyroid arteries in goiter, quoting Gutknecht,[3] Jores,[4] Isenschmid,[5] Hesselberg [6] and Wangensteen.[7] Gutknecht observed extensive hyalinization and calcification in blood vessels in thyroid glands, including capillaries. Jores described granular calcareous deposits associated with fragmentation of the internal elastic lamella in arteries of goitrous thyroid glands. Arteries so involved also presented a thickened intima. This involvement was independent of generalized arteriosclerosis. Hesselberg and Isenschmid noted arteriosclerosis in the thyroid glands of infants, the former in a new-born infant. Wangansteen found intimal thickening and calcification and splitting of the internal elastic coat to be of not infrequent occurrence in goiters of cretins. Karsner [8] stated that arteriosclerosis is common in goiter, often independent of arteriosclerosis in other parts of the body.

The present study was undertaken in an attempt to determine the incidence of calcification of the internal elastic lamella in goitrous thyroid glands and the relationship of associated factors that might be concerned in its occurrence. Interest concerning the significance of the lesion had been aroused by observing its presence in several thyroid glands removed surgically, while it had been observed rarely in other organs and tissues.

The lesion, although varying in degree in different cases, was of fairly constant morphology and distinct from ordinary arteriosclerosis. In all instances it was manifest only on microscopic study. The least

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From the Department of Pathology, Medical College of Virginia.

1. Curtis, in discussion on Phemister and Delaney: Arch. Path. **9**:957, 1930.
2. Curtis and Delaney: Arch. Path. **10**:580, 1930.
3. Gutknecht: Virchows Arch. f. path. Anat. **99**:419, 1885.
4. Jores: Beitr. z. path. Anat. u. z. allg. Path. **21**:211, 1897.
5. Isenschmid: Frankfurt. Ztschr. f. Path. **5**:205, 1910.
6. Hesselberg: Frankfurt. Ztschr. f. Path. **5**:322, 1910.
7. Wangensteen: Surg., Gynec. & Obst. **48**:613, 1929.
8. Karsner: Human Pathology, Philadelphia, J. B. Lippincott Company, 1931, p. 853.

conspicuous and presumably earliest lesions were confined to hyalinization or calcification of portions of internal elastic membrane, with fragmentation; these were sufficiently insignificant to be overlooked readily. With increase in calcareous deposition, the convoluted although fragmented contour of the elastica became irregular in thickness or assumed a beaded appearance. In more advanced cases, with extension of the deposits into the media and into the now somewhat thickened intima, the condition was indistinguishable from Mönckeberg's [9] medial calcification of peripheral arteries. In the earlier lesions, degenerative changes in the media immediately adjacent to the calcified portions of the elastic membrane were manifest in vacuolation and fibrillar lysis. Intimal thickening, seldom conspicuous and without plaque formation, limited to fibrillar proliferation, always had the aspect of being secondary. The media in some instances was slightly or moderately hypertrophied.

MATERIAL AND PROCEDURE

The material on which the bulk of this study is based consisted of the cases of goiter represented by 100 consecutive partial thyroidectomies. Multiple sections of each specimen were prepared, selection of blocks being made largely in reference to visible capsular blood vessels. The average number of sections per case was 5; the extremes were 2 and 22. Sections in selected cases were treated with 1 per cent silver nitrate—von Kossa's method for demonstrating calcium. In studying the sections, particular attention was given to arteries, any deviation from normal being recorded. The condition of veins also was noted, and the presence and character of other lesions of the thyroid gland were reported. In parallel columns, the patient's age, blood pressure, duration of symptoms, functional thyroid state and use of iodine therapy, and the presence of any concomitant chronic disease other than goiter, were tabulated.

Twenty-five nongoitrous and essentially normal thyroid glands of children and adults, derived from routine autopsies, and 50 thyroid glands of fetuses and new-born infants were studied similarly with a view to comparison of the occurrence of the lesion in goiter.

OBSERVATIONS

The observations are summarized in tables 1 and 2. Calcification and fragmentation of the arterial internal elastic membrane occurred in 56 of the 100 cases. The morphology and variations of the lesion

9. MacCallum, W. G.: A Text-Book of Pathology, Philadelphia, W. B. Saunders Company, 1931, p. 326.

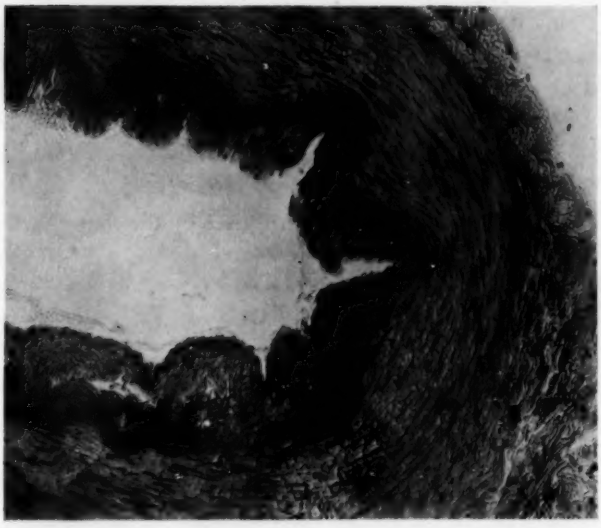

Fig. 1.—A relatively early stage of the lesion. Portions of the internal elastic membrane are calcified. There are moderate degrees of intimal proliferation and medial hypertrophy.

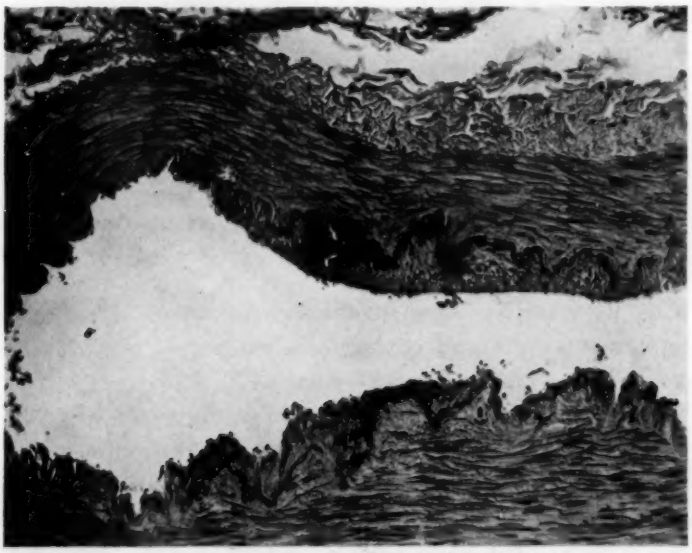

Fig. 2.—An artery of an otherwise normal thyroid gland. A threadlike calcareous band occupies much of the internal elastic membrane, with more bulky calcareous deposition in some portions. Intimal proliferation, focal medial degeneration and medial hypertrophy are associated.

coincide with the description given in an earlier paragraph. In somewhat less than half the cases, the lesion was insignificant, often confined to a threadlike line of calcification along a portion of the internal elastic membrane. In over half of the instances the lesion was prominent, and in a few cases was seen as bulky, calcareous masses occupying most of the thickness of the arterial wall and effecting a structure identical with Mönckeberg's type of medial calcification. Intermediate degrees of involvement filled gaps in a gradual transition from the earliest to the most advanced lesions. Perhaps the development of Mönckeberg's sclerosis is traced here. Limited fibrillar connective tissue proliferation in the intima and varying degrees of medial degeneration or hypertrophy were invariably associated. By far most of the examples of the lesion occurred in arteries of good size lying in the capsule of the gland.

Fig. 3.—An advanced stage, compatible with Mönckeberg's medial calcification, occurring in a diffuse nonhyperplastic goiter.

If the 100 goiters are divided into 5 classes corresponding to morphologic types, and each class is subdivided into two groups—those which include the lesion and those which do not (table 1)—it is found that the lesion has no definite predilection for any type of goiter; the incidence is distributed fairly proportionately over the different classes. Diffuse nonhyperplastic goiter, nodular hyperplastic goiter and neoplasms of the thyroid gland are represented by numbers of cases too small for reliable deductions. Former accounts of the occurrence of

TABLE 1.—*Observations in 100 Goitrous Thyroid Glands in Relation to Calcification of the Arterial Internal Elastic Coat*

Type of Goiter	Group	Cases	Percentage	Average Age of Patient, Yr.	Average Duration of Goiter, Yr.	Average Blood Pressure	Cases with Hyperthyroidism	Cases with Iodine Therapy	Cases with Concomitant Chronic Disease	Other Lesions of Thyroid Gland
Diffuse Nonhyperplastic	Lesion present	6	75	34.0	3.2	130/72	1	3	3: chr. inflam. foci, dent. caries	Nothing irrelevant to type of goiter
	Lesion absent	2	25	23.0	0.7	120/75	1	2	2: chr. inflam. foci	Nothing irrelevant to type of goiter
Nodular Nonhyperplastic	Lesion present	14	50	40.0	8.2	137/86	2	5	7: chr. inflam. foci, ut. fibroids, cardiac hypertr.	Nothing irrelevant to type of goiter
	Lesion absent	14	50	40.0	10.6	136/77	3	5	8: chr. inflam. processes, ut. fibroids	Nothing irrelevant to type of goiter
Diffuse Hyperplastic	Lesion present	25	57	34.6	1.8	132/71	25	25	13: chr. inflam. foci, gastric ulc. tb., syph., rheum.	Marked chr. thyroiditis, 2 instances
	Lesion absent	19	43	31.6	1.4	137/72	19	19	12: chr. inflam., cardiovasc. dis., ut. malposition	Marked chr. thyroiditis, 1 instance
Nodular Hyperplastic	Lesion present	4	44	37.0	5.6	159/86	4	3	2: chr. inflam. processes	Nothing irrelevant to type of goiter
	Lesion absent	5	56	39.0	9.8	161/89	5	4	3: chr. inflam. processes	Nothing irrelevant to type of goiter
Neoplasm of Thyroid	Lesion present	7	64	37.4	4.2	152/95	0	0	3: pelvic inflam. dis., toxem. of preg., dent. caries	Fibrosis, atrophy, inflam. resp.
	Lesion absent	4	36	33.2	5.0	137/73	0	0	3: diabetes, chr. inflam. foci, syphilis	Fibrosis, atrophy, inflam. resp.

TABLE 2.—*Occurrence of Calcification of Internal Elastic Membrane in Goitrous and in Nongoitrous Thyroid Glands*

	Cases	Number with Arterial Lesion	Percentage with Arterial Lesion
Goitrous thyroid glands	100	56	56
Nongoitrous thyroid glands from routine autopsies, children and adults	25	3	12
Thyroid glands of fetuses and new-born infants	50	0	0

arteriosclerosis in goiter have emphasized its being found in nodular (adenomatous) goiter. In the present study, this group contributed the lowest percentage of occurrence. The least frequent occurrence of the lesion was found in nodular hyperplastic goiter, the lesion being present in 4 cases out of 9 (44 per cent), while the most frequent occurrence was found in the type represented by the smallest number of cases, i. e., diffuse nonhyperplastic (colloid) goiter—the incidence here being 6 out of 8, or 75 per cent. Half of the cases of nodular nonhyperplastic (adenomatous) goiter, 25 of 44 cases of diffuse hyperplastic (exophthalmic) goiter, and 7 of 11 neoplasms of the thyroid gland presented one or more instances of the lesion.

The ages of patients presenting the lesion and of those not presenting it were too close for any deductions in this regard. It was seen to occur at an age younger than that at which appreciable sclerosis is to be expected. The youngest patient in whom it occurred was a young colored woman, 20 years of age, who had diffuse hyperplastic goiter.

The average blood pressure of those having the lesion was about the same as that of those not having it.

The lesion bore no constant relationship to the duration of goiter, to the presence of hyperthyroidism or to iodine therapy. Concomitant chronic disease other than goiter was as frequent and as varied in nature in those having the lesion as in those not having it. The structure of the thyroid gland other than the arterial lesions presented no appreciable differences in the two groups.

In 3 of 25 nongoitrous, essentially normal thyroid glands removed at autopsy, the calcareous arterial lesion was found, and was morphologically identical with the lesion in goiter. The lesion was not observed in sections of arteries of other viscera in these cases. One of these cases was that of a white man, 54 years of age, dying from carbon monoxide poisoning; another, that of a colored man, 37 years old, with intestinal obstruction, and the third, that of a 62 year old colored man, who had a ruptured syphilitic aneurysm.

I believe the occurrence of calcification of the arterial internal elastic membrane in the normal thyroid gland in adults to be higher than this 12 per cent would indicate. The relatively small series included several infants and children, and the average number of sections studied in each case is smaller than that in the goiter series, owing largely to lack of available material.

Hesselberg's observation of arteriosclerosis in the thyroid gland of a new-born infant carries the suggestion that possibly the arterial lesion is congenital. I have studied sections of the thyroid glands of 50

fetuses and new-born infants without finding the lesion; a normal vascular structure was constant throughout.

SUMMARY AND COMMENT

An arterial lesion of the thyroid gland, characterized by fragmentation, hyalinization and calcification of the internal elastic lamella, is described. In more advanced cases it conforms with Mönckeberg's medial calcification of peripheral arteries. It occurred in 56 of 100 goiters, irrespective of the age of the patient, blood pressures, duration of the goiter, hyperthyroidism, iodine therapy, presence of concomitant disease or structure of the thyroid gland. It is found also in the normal thyroid gland, but probably less frequently. It was not found in a series of 50 thyroid glands of fetuses and new-born infants. It is rare in tissues other than the thyroid. The significance of the lesion is not manifest; morphologically, it is degenerative.

PATHOLOGY OF SHOCK

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Throughout this discussion the term shock will be used to designate the condition following severe physical injury in which low blood pressure, rapid, weak pulse, decreased blood volume, increased concentration of blood and usually rapid respiration and decreased temperature are prominent features. It will be used as synonymous with the terms circulatory shock, surgical shock, wound shock, traumatic shock or secondary shock, referring to the condition that develops some hours following injury as distinct from that occurring immediately on receipt of injury.

Space does not permit a review of the subject. Adequate reviews are available.[1] A widely accepted view is that shock results from traumatic toxemia; that effective blood volume is reduced by stagnation of blood in dilated peripheral capillaries and venules, and that such withdrawal of blood from currency results in embarrassment or failure of the circulatory function.

Recent experiments have been interpreted to indicate that traumatic toxemia is not a factor, but that shock results from hemorrhage and loss of fluid into the area of trauma. The experiments of Blalock and his associates[2] included intricate combinations of resection of one or both femurs, the application of tourniquets with and without obstruction to one or both femoral vessels, and trauma accompanied by extensive hemorrhages. These were made under barbital anesthesia, which alone fre-

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1. Cannon, W. B.: Traumatic Shock, Philadelphia, D. Appleton and Company, 1923. Krogh, A.: The Anatomy and Physiology of Capillaries, ed. 2, New Haven, Conn., Yale University Press, 1929.

2. Blalock, A.: Arch. Surg. **15**:762, 1927; **20**:959, 1930; **22**:314, 598 and 610, 1931. Blalock, A., and Bradburn, H. B.: Arch. Surg. **19**:725, 1929; **20**:26, 1930. Johnson, G. S., and Blalock, A.: Arch. Surg. **22**:626 and 855, 1931. Blalock, A.; Beard, J. W., and Johnson, G. S.: J. A. M. A. **97**:1794, 1931. Beard, J. W., and Blalock, A.: Arch. Surg. **22**:617, 1931. Butler, Virginia; Beard, J. W., and Blalock, A.: Arch. Surg. **23**:848, 1931. Harris, P. N., and Blalock, A.: Arch. Surg. **22**:638, 1931.

quently produces shocklike phenomena.[3] Blalock recorded that in four of five normal dogs there was from 56 to 110 mm. fall in blood pressure from barbital alone. One normal dog died five hours after the administration of barbital.[4] The viscera showed congestion, edema and capillary hemorrhages such as Krogh [5] describes as the characteristic effects of capillary poisons. In a maze of experimental intricacies, using barbital anesthesia and variation of blood pressure as the criterion of shock, the authors lost all distinction between shock and hemorrhage. They concluded that the cause of low blood pressure after various types of injury was the loss of whole blood or of plasma at the site of the trauma.

Parsons and Phemister [6] drew similar conclusions from experiments in which trauma, under barbital anesthesia, produced hemorrhages of from 300 to 650 cc. The experiments were not designed to distinguish between shock and hemorrhage.

Robinson and Parsons [7] compared the water content of the blood and muscles of dogs. Following hemorrhage, the muscles lost and the blood gained in water content. Following administration of histamine, the muscles gained and the blood lost in water content. Following mild trauma under barbital anesthesia there was a transfer of water from the blood to the muscles as in dogs treated with histamine. These dogs were then subjected to repeated trauma accompanied by hemorrhage, after which a transfer of water from the tissues to the blood occurred. They concluded that loss of blood into traumatized areas causes shock. The conclusion appears contrary to the evidence obtained from the mildly traumatized animals before hemorrhage was made a factor. To produce further trauma and hemorrhage in animals already suffering from shock only introduced confusion.

The opinion that shock is due to hemorrhage might best be tested by comparing the effects of trauma without hemorrhage with those of hemorrhage alone. In each of the experiments mentioned, the shocklike effects of barbital apparently were not considered.

TISSUE CHANGES IN EXPERIMENTAL SHOCK

Valuable evidence in the question of trauma vs. hemorrhage as factors in shock may be derived from the appearances of the tissues in those conditions. The ischemia of tissues in death from hemorrhage

3. Peterson, Frederick; Haines, W. S., and Webster, R. W.: Legal Medicine and Toxicology by many Specialists, ed. 2, Philadelphia, W. B. Saunders Company, 1923, vol. 2, p. 661.

4. Blalock, A.: Arch. Surg. **22**:604, 1931.

5. Krogh, A.: The Anatomy and Physiology of Capillaries, ed. 2, New Haven, Conn., Yale University Press, 1929, p. 195.

6. Parsons, Eloise, and Phemister, D. B.: Surg., Gynec. & Obst. **51**:196, 1930.

7. Robinson, William, and Parsons, Eloise: Arch. Path. **12**:869, 1931.

is well recognized. The opposite changes have been noted in shock from trauma in human beings and in experimental shock. Morrison and Hooker,[8] Gasser, Erlanger and Meek,[9] Keith,[10] Bayliss,[11] Cannon, Fraser and Hooper,[12] Whipple, Smith and Belt [13] and others have recorded observations on congestion, capillary hemorrhages and edema in shock. Dale, Laidlaw and Richards [14] found exactly these changes following administration of histamine. Krogh [5] found such effects resulting from many capillary poisons. Apparently sufficient emphasis has not been given to these visible changes.

In the experiments reported in this paper, shock was produced in dogs, primarily to secure tissues for study. Incidentally, these experiments have some bearing on the etiology. In shock the blood becomes concentrated. It is common knowledge that dilution of blood occurs following hemorrhage. The averages of the red cell count, hemoglobin percentage and specific gravity of the blood for a period of from five to ten days prior to the experiments were compared with variations noted during and following the experiments. When records of the blood pressure were made, morphine, 0.2 mg. per kilogram of body weight, was given hypodermically. Then, under local procaine hydrochloride anesthesia, an arterial cannula was inserted and connected with a mercury manometer. No prolonged continuous records were attempted.

Procedures were devised to produce shock without local injury and hemorrhage. A normal dog was killed under ether anesthesia. Quantities of muscle were excised immediately and ground through a meat chopper. This, diluted with an equal quantity of physiologic solution of sodium chloride, was sterilized by autoclave and refrigerated until used.

Freshly ground, unsterilized muscle was extracted with an equal volume of distilled water. The fluid was squeezed out through gauze and centrifugated at high speed for fifteen minutes. The layer of fat from the top and the sediment from the bottom were discarded. Sodium

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8. Morrison, R. A., and Hooker, D. R.: Am. J. Physiol. **37**:86, 1915.

9. Gasser, H. S.; Erlanger, J., and Meek, W. J.: Am. J. Physiol. **49**:90 and 151, 1919; **50**:31, 1919.

10. Keith, N. M.: Medical Research Committee, Special Report Series, no. 26, 1919, p. 36.

11. Bayliss, W. M.: Medical Research Committee, Special Report Series, no. 25, 1919, p. 26; no. 26, 1919, p. 24.

12. Cannon, W. B.; Fraser, John, and Hooper, A. N.: Medical Research Committee, Special Report Series, no. 25, 1919, p. 72; J. A. M. A. **70**:526, 1918.

13. Whipple, G. H.; Smith, H. P., and Belt, A. E.: Am. J. Physiol. **52**:72, 1920.

14. Dale, H. H.; Laidlaw, P. P., and Richards, A. N.: Medical Research Committee, Special Report Series, no. 26, 1919, p. 8; Brit. M. J. **1**:381, 1917; J. Physiol. **52**:110, 1918; 335, 1919.

chloride was added to render the fluid isotonic. It was passed through paper filters, sterilized by Berkefeld filtration, and stored in refrigeration. A watery extract of traumatized muscle was similarly prepared from a dog in which shock had been produced by bruising.

Dog S-24.—Under surgical asepsis, 200 Gm. of sterilized normal muscle was introduced into the peritoneal cavity of an adult male mongrel weighing 6.32 Kg. The operation was done under ether anesthesia lasting twenty minutes. The dog recovered well from the operation. He was active and even playful. Presently he refused all food, but drank water freely. He appeared acutely ill, refused to move about and shivered frequently. There were frequent bowel movements, and the feces contained blood-tinged mucus. The extremities became cold to the touch; respirations and pulse were increased to twice their normal rates. He died ten and one-half hours following the operation. Changes in the concentration of the blood were as shown in table 1.

After death there was about 150 cc. of thin, blood-tinged fluid in the peritoneal cavity. The peritoneum was intensely congested, and the blood vessels along the

TABLE 1.—*Changes in Concentration of Blood of Dog S-24*

Date	Hour	Specific Gravity	Hemoglobin Percentage	Red Cells	
(Average normal).....		1.059	98	5,820,000	
3/24/32	6:30 a.m.	1.060	101	5,110,000	
	7:00 a.m.	Operation			
	10:30 a.m.	1.065	94	4,860,000	
	1:30 p.m.	1.065	104	5,694,000	
	3:20 p.m.	1.067	108	5,695,000	
	4:50 p.m.	1.075	120	6,400,000	
	5:15 p.m.	1.075	130+	7,450,000	Death

mesenteric attachment were markedly distended. The bowels contained bloody mucus. Congestion was marked in the mucosa of the bowels and in the liver, kidneys, spleen, lungs and myocardium. Petechial hemorrhages were present in the lungs and in serous surfaces.

Microscopic examination showed numerous hemorrhages associated with marked congestion of the capillaries and venules in each of the organs mentioned (fig. 1).

Four other dogs similarly treated showed features identical with those described. The data concerning these are summarized in table 2. Evidently some substance capable of producing rapidly a fatal shocklike condition is absorbed from sterile muscle implanted in the peritoneal cavity. There was no loss of blood by hemorrhage in these experiments.

Sterile aqueous extract of normal muscle produced similar effects in dog S-11 when introduced intraperitoneally. It produced an immediate fall in blood pressure when introduced intravenously in the following experiments:

Dog S-8.—An adult male mongrel, weighing 21.3 Kg., was connected with a mercury manometer. An intravenous injection of 20 cc. of watery extract of

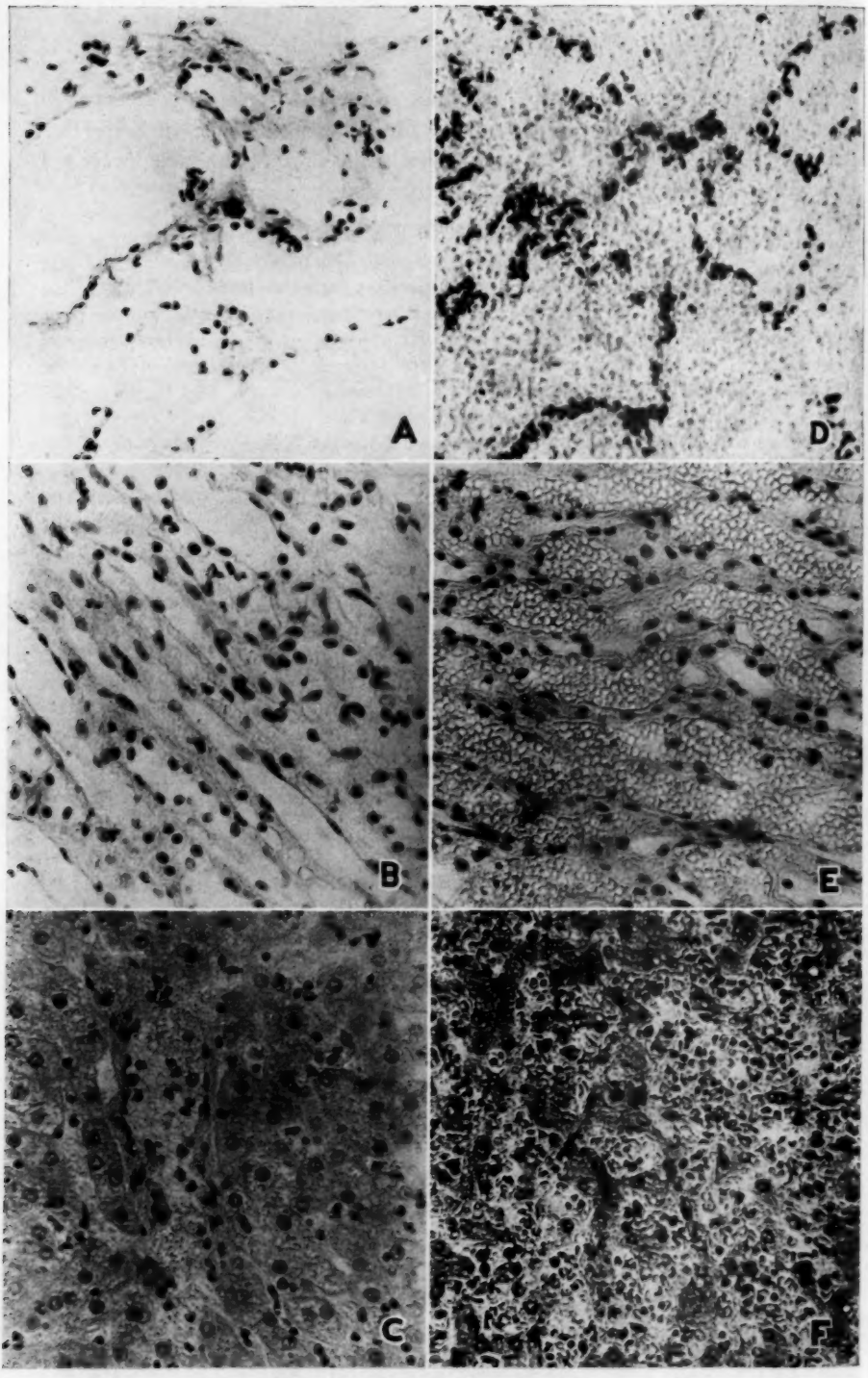

Fig. 1.—*A*, *B* and *C* are photomicrographs of lung, kidney and liver following death produced by repeated hemorrhages within forty-eight hours. *D*, *E* and *F* are photomicrographs of lung, kidney and liver following death in fifty-two hours after trauma to muscles.

normal muscle caused the blood pressure to fall 110 mm. During the following two hours the pressure gradually rose 64 mm. The animal recovered.

Dog S-9.—The sterile filtrate from 25 Gm. of normal muscle was incubated for twenty hours to permit action of such ferments as it might contain. In a dog weighing 10.25 Kg., intravenous injection of 10 cc. of the filtrate caused the blood pressure to decline 56 mm. The remainder of the filtrate was given in three injections at intervals during an hour. This caused the blood pressure, 140 mm. before the experiment, to drop to 44 mm.—a fall of 96 mm. During eighteen hours the pressure rose to 110 mm. and recovery might have followed. Since it was desired to observe tissue changes, the dog was killed painlessly. The lungs were congested and mottled with numerous dark red areas, which microscopic examination showed to be due to extravasation of blood from capillaries. There was moderate edema of the lungs. The kidneys were markedly congested; otherwise the organs appeared normal.

Dog S-14.—A sterile filtrate of traumatized muscle was injected intravenously into a male mongrel weighing 7.28 Kg. Seven injections of 25 cc. each were made at intervals during a period of two hours. This produced marked disturbances of the pulse, respiration and general condition. Fifteen hours later the animal did not react to stimuli and was very ill, with respirations 40 per minute, pulse imperceptible and extremities cold. The hemoglobin had risen from 80 to 105 per cent, and the erythrocytes from 5,400,000 to 6,840,000—about 25 per cent increased concentration of the blood. There were frequent evacuations of blood-tinged mucus from the bowels. The animal died in twenty-one hours.

The gross and microscopic changes were identical in character with those of dog S-24. The congestive features were most marked, and there was widespread central necrosis in the hepatic lobules.

For other similar experiments, see table 2. The effect of muscle extract on these dogs closely resembled that which may follow administration of histamine.

The following experiment illustrates the effects of hemorrhage on the composition of the blood and on the tissues of the body.

Dog S-23.—The dog was a female mongrel weighing 10.46 Kg. For six days prior to the experiment, the hemoglobin percentage, red cell count and specific gravity of the blood were determined (table 3).

It will be seen that a loss of 700 cc. of blood within thirty-six hours was sustained by a dog of small size. Assuming the blood to have an average specific gravity of 1.050, this amounted to 7 per cent of the body weight or about 70 per cent of the total blood volume. Such a loss of blood reduced the hemoglobin only to 36 per cent and the specific gravity to 1.032. At this time, the dog showed no evidence of illness. It was active and playful as normally, and undoubtedly would have recovered. It required a further loss of 400 cc. of blood to cause death.

After death there was marked anemia of all the organs and tissues. The lungs were very pale throughout and showed neither congestion nor areas of hemorrhage (fig. 2). The heart muscle was pale brown. There were no petechiae in the endocardium or elsewhere. The spleen, liver and kidneys were dry on section and showed no congestion. All the peritoneal surfaces were pearly white. The venules along the mesenteric attachment were not visible. The mucosa of the entire gastro-intestinal tract was dull white. On microscopic examination, all the tissues had a normal appearance except that there was less blood present than normally (fig. 1).

TABLE 2.—*Summary of Experiments Not Described in the Text*

Dog	Treatment	Change in the Blood	Result	Capillary Congestion	Edema	Capillary Hemorrhage
S-7	Filtrate of traumatized muscle, 175 cc., intravenously injected	No data	Died in 16 hr.	Lungs, bowel, cardiac muscle, kidney, liver	Lungs	Lungs, kidney, liver
S-11	Filtrate of normal muscle, 275 cc., intraperitoneally injected	Increased concentration	Killed in 24 hr.	Peritoneum, intestinal mucosa, liver, lungs	Peritoneal cavity, submucosa	Endocardium, mucosa of bowel, renal cortex
S-13	Filtrate of normal muscle, 235 cc., intravenously injected	Increased concentration	Died in 23 hr.	Peritoneum, intestinal mucosa, spleen	Intestinal mucosa	Endocardium, spleen
S-3	Normal muscle fresh, 40 Gm., intraperitoneally injected	No data	Died in 16 hr.	Lungs, spleen, lymph node, kidney, liver, peritoneum, myocardium, gastro-intestinal tract	Peritoneum	Lungs, kidneys
S-18	Sterile muscle substance, 350 Gm., intraperitoneally injected	No data	Died in 17 hr.	Lungs, liver, kidney, peritoneum, gastro-intestinal tract	Peritoneum, liver, skeletal muscle	Endocardium, lungs, kidney, gastro-intestinal mucosa
S-21	Sterile muscle, 200 Gm., in peritoneum	Increased concentration	Died in 12 hr.	Lungs, liver, spleen, myocardium, kidney	Lungs, skeletal muscle	Lungs, liver, kidney
S-22	Sterile muscle, 100 Gm., in peritoneum	Increased concentration	Killed in 32 hr.	Lungs, liver, myocardium, kidney	Skeletal muscle	Lungs, kidney
S-6	Trauma to muscles of thigh	Decreased concentration, 11 per cent	Died in 32 hr.	Lungs, gastro-intestinal tract, myocardium, liver, kidney	Lungs, submucosa of bowel	Pleura, endocardium, respiratory mucosa, lungs
S-12	Trauma to muscles of thigh	Increased concentration	Died in 22 hr.	Lungs, liver, peritoneum, gastro-intestinal tract, myocardium	Lungs, respiratory mucosa, mediastinum	Endocardium, lungs, kidneys, mucosa of bowel

* The same gross and microscopic changes were present as described for dog S-24.

TABLE 3.—*Changes in Concentration of Blood Following Hemorrhage*

Date	Hour	Specific Gravity	Hemoglobin Percentage	Red Cells	Blood Withdrawn, Cc.	
(Average normal).....		1.056	92	5,200,000	...	
3/15/32	11:00 a.m.	1.055	98	5,400,000	100	
	3:00 p.m.	1.055	97	6,320,000	100	
	5:00 p.m.	1.055	95	5,300,000	...	
3/16/32	10:00 a.m.	1.055	80	5,400,000	100	
	11:30 a.m.	1.050	75	4,910,000	100	
	1:00 p.m.	1.047	57	4,400,000	100	
	2:30 p.m.	1.042	60	3,890,000	100	
	4:15 p.m.	1.037	49	3,420,000	100	
3/17/32	9:00 a.m.	1.032	36	1,380,000	...	
	9:30 a.m.	.....	..	.....	400	Death

We wish to emphasize the contrast between the findings in this animal and those that accompany the shock syndrome.

The effects of barbital are shown by the following experiment:

Dog S-19.—A vigorous adult male, weighing 13.6 Kg., was given 4 Gm. of sodium barbital intravenously. This is the dosage, 0.3 Gm. per kilogram, used by several authors of articles on shock. Three hours later, the blood pressure had declined 94 mm., and the pulse and respirations were twice their former rates. Beginning at this time, 100 cc. quantities of blood were withdrawn at intervals of thirty minutes. The dog died following the loss of 500 cc. of blood—less than 4 per cent of the body weight.

The lungs were mottled with areas of congestion. There were numerous petechial hemorrhages in the endocardium. There was congestion of the liver, spleen, kidneys and gastro-intestinal mucosa, notwithstanding the fact that death was caused by hemorrhage.

Fig. 2.—Lungs of dogs S-23 (death from repeated hemorrhages within forty-eight hours) and S-24 (shock from muscle substance in the peritoneal cavity).

Barbital alone causes marked fall in blood pressure, increased pulse and respiratory rates, and inability to recover from hemorrhage. The tissue changes and physiologic effects of barbital resemble those of shock.

In the next experiment, shock was produced by trauma:

Dog S-25.—At 1:15 p. m., an adult mongrel male, weighing 10.47 Kg., was anesthetized with ether, and the muscles of the hindlegs were severely bruised. As the dog returned to consciousness, pain was prevented by hypodermic injections of morphine, 0.5 mg. per kilogram of body weight at intervals. The dog refused food, but drank water. On the following day, he appeared normal except for marked swelling of the thighs. He was given ether, and the muscles were retraumatized moderately. On March 24, his condition was about the same as on the 23rd. Following heavy massage under light ether anesthesia, a condition of collapse rapidly developed. The pulse and respirations became rapid and the

extremities cold. He became unresponsive to stimuli and died two hours later. The changes in the blood are shown in table 4. Note that at one period there was a decrease in concentration of the blood. This would indicate that hemorrhage was perhaps a factor.

In dogs treated with sterile filtrate of normal muscle, with filtrate of traumatized muscle, with sterile muscle substance implanted in the peritoneal cavity and with mechanical trauma, similar physiologic disturbances resulted. Tissue changes of an identical nature were found at autopsy. These changes included widespread capillary dilatation, especially marked in the lungs, the gastro-intestinal tract, the liver and the kidneys. There were numerous capillary hemorrhages, and edema was present frequently. A prominent antemortem feature was increased concentration of the blood.

TABLE 4.—*Changes in Concentration of Blood of Dog S-25*

Date	Hour	Specific Gravity	Hemoglobin Percentage	Red Cells	
(Average normal).....		1.058	94	5,840,000	
3/22/32	4:30 p.m.	1.058	101	6,000,000	
3/23/32	10:30 a.m.	1.060	99	5,420,000	
	1:00 p.m.	1.057	101	6,150,000	
	4:30 p.m.	1.055	90	5,900,000	
3/24/32	8:00 a.m.	1.053	86	3,800,000	
	1:30 p.m.	1.054	60	4,100,000	
	4:30 p.m.	1.057	70	4,500,000	
	7:30 p.m.	1.064	96	5,900,000	
	8:00 p.m.	1.065	99	5,900,000	Death

OBSERVATIONS IN HUMAN CASES OF SHOCK

In human cases apparently of shock, we have found the same capillary phenomena as in the experiments on dogs.

A white woman, aged 65, was operated on for the removal of an ovarian tumor. Ether was used, and the anesthesia lasted thirty-five minutes. The operation, which was complicated by the presence of adhesions, lasted twenty-five minutes. No significant hemorrhage occurred, and the condition was recorded as fair at the close of the operation. Collapse developed. The pulse became rapid and feeble, the skin pale, the extremities cold and the respirations 30 to 40 per minute. She was given 600 cc. of saline solution intravenously and caffeine and atropine hypodermically. Heat was applied, and further stimulation was attempted with drugs. These were ineffective. The blood pressure fell to 80 mm. systolic and 70 mm. diastolic. She became progressively weaker and died within six hours. There was no evidence of gross hemorrhage. About 2 liters (2,000 cc.) of thin, blood-tinged fluid was present in the peritoneal cavity. The entire intestinal tract was relaxed, atonic and markedly congested. The lungs were dark red and contained a large amount of fluid. The left lung weighed 310 Gm., and the right, 490 Gm. There were concretions in the gallbladder, and the kidneys were arteriosclerotic. No other significant changes were present.

On microscopic examination, the alveoli of the lungs contained variable amounts of serous fluid; some were filled, other partially filled, and others contained air. The capillaries were widely distended with corpuscles. No gross hemorrhages were present, but many corpuscles had escaped into the alveolar spaces. The capillaries of the mucosa of the bowel were distended, and the submucosa was edematous.

Permission was granted by Surgeon General Patterson to study material collected by the medical service of the American Expeditionary Forces in France. Accordingly Major P. E. McNabb, curator of the Army Medical Museum, submitted twenty-six postmortem records selected as those of probable cases of shock. Records of cases of injury to the brain or cord and of cases of penetration of the chest or abdomen were rejected. There remained twenty-two records of multiple lacerated shell-fragment wounds of the arms, legs, shoulders or buttocks. In each of twenty cases several sections of lung were available for study. The cases fell into groups as follows:

Group 1 consisted of eight cases in which no gas bacillus infection occurred, and in which hemorrhage was a minor factor. The following record (accession no. 4259) is representative of this group:

A soldier had suffered extensive lacerated shell wounds of both feet, with comminuted fractures of the feet and legs. He was brought in with a tourniquet about each leg. Heat was applied, and hot fluids were given by mouth. The tourniquets were released. Five hours later the blood pressure was 70 mm. systolic and 58 mm. diastolic. Gum acacia solution 750 cc. given intravenously raised the blood pressure 13 points. His condition did not improve, and he died in shock eight hours after admission, although no hemorrhage had occurred in the interim.

Postmortem examination showed marked posterior lividity, edema of the brain and marked venous engorgement of the viscera. Both lungs were heavy and livid red, and the crepitation was diminished. They contained numerous areas of petechial hemorrhage. Blood and fluid in increased amount flowed from the surfaces made by sectioning. There were petechial hemorrhages in the endocardium. On microscopic examination, the lungs showed marked capillary engorgement, numerous areas of capillary hemorrhage and moderate diffuse edema.

Group 2 embraced five cases of injuries similar to those in group 1, but complicated by varying degrees of gas bacillus infection. In each case, the record showed venous engorgement, marked pulmonary hyperemia, marked edema of the lungs and petechial hemorrhages in the lungs or in serous surfaces. On microscopic examination, the sections of lung showed no difference from those in group 1.

Group 3 was composed of four cases in which moderate acute anemia from hemorrhage was present. Gas infection was present in one of these. In two cases, the lungs had normal consistence and crepitation. Microscopically there were edema, congestion and capillary hemorrhages. Anemia and the shock mechanism appeared to be combined. The other two cases showed no essential differences from those described under group 1. Grossly and microscopically, the lungs showed congestion, edema and capillary hemorrhages in marked degree. Ischemia was not present. The case in which gas infection was present showed no gross or microscopic differences from those of traumatic shock alone.

Group 4 included five cases in which hemorrhage seemed a major factor. Gas infection was present in one of these. The following case (accession no. 4344) is representative of four in this group:

A soldier had experienced a shell fragment injury to the right lumbar region. He was admitted in extreme shock and given the usual treatment for shock. The time of injury and of death were not recorded. At postmortem examination there was very slight posterior lividity. Free blood, 1,500 cc. in amount, was present in the peritoneal cavity. The lungs varied in color from light gray to light pink. The crepitation was normal. The surfaces made by sectioning gave up a smaller amount of fluid than normally. On microscopic examination, the sections of the lungs contained little visible blood, and there was no edema or areas of capillary hemorrhage.

Three other cases showed similar features. The viscera were pale and anemic. "Dry autopsy" was noted in the records. Gross and microscopic examinations of the lungs showed less blood than normally. The examinations indicated that hemorrhage was the chief cause of death. In the fourth case, gas bacillus infection was present. The patient lived three days following injury, and no hemorrhages occurred in the interim. The gross and microscopic features were those of shock.

Summarizing the observations made at the time of autopsy in the seventeen cases in groups 1, 2 and 3 in which shock was a major factor, one finds marked pulmonary congestion and edema in sixteen cases, petechial hemorrhages in serous surfaces in eight cases and visceral venous engorgement in eleven cases.

Microscopic study of sections of the lungs showed marked capillary congestion with varying degrees of capillary hemorrhage and edema in fifteen cases in this series. In two cases no sections were available.

It is noteworthy that the changes present in these cases are not characteristic of anemia from hemorrhage, and that they were absent in the cases of group 4, in which hemorrhage was assigned as the chief cause of death.

COMMENT

Most conditions of disease are accompanied by tissue changes which are more or less characteristic, and which both aid in the recognition of the cause and corroborate the correct etiologic interpretation. Shock is not, as has been supposed, an exception to the general rule.

A significant feature of shock is increased concentration of the blood. This has been mentioned frequently in previous reports. That hemorrhage is often a complicating factor of most serious gravity in shock is generally admitted. Trauma of sufficient severity to produce shock in dogs is accompanied by varying degrees of hemorrhage. In one traumatized animal, S-6, a decrease of 11 per cent in the concentration of the blood was observed. The death of this dog may have been due to a combination of shock and hemorrhage. That death was not due to hemorrhage alone was indicated by the marked congestion, edema and numerous capillary hemorrhages in the viscera. Those who have had

difficulty in drawing distinctions might have observed whether the blood and tissues of shocked animals showed the changes that serious hemorrhage will produce.

We have noted that edema, particularly of the lungs, is more marked in cases of shock in man than in experimental shock in animals.

CONCLUSIONS

The shock syndrome is accompanied by gross and microscopic changes opposite in character to those produced by hemorrhage. The changes include dilatation and engorgement of capillaries and venules, permeability of capillary walls, as indicated by petechial hemorrhages and edema, and frequently effusion into serous cavities. Increased concentration of the blood is a characteristic phenomenon in shock.

These vascular phenomena are widespread, but are most prominent in the pulmonary and gastro-intestinal tracts.

These circulatory effects can be produced by trauma, by intravenous or intraperitoneal injections of extract of normal muscle or by implanting muscle substance into the peritoneal cavity. This tends to corroborate the view that products absorbed from injured tissues produce the shock syndrome, and that these act by causing dilatation and increased permeability of the capillaries and venules.

Barbital produces physiologic and histologic changes resembling those of shock. Drugs having such properties should not be used when normal blood pressure and capillary tonus are important.

Laboratory Methods and Technical Notes

PRESERVATION OF THIN SECTIONS OF TISSUE IN NATURAL COLORS

JAMES E. DAVIS, M.D., AND ARTHUR L. AMOLSCH, M.D., DETROIT

Satisfactory preservation of valuable specimens has always been a problem. The complete specimen preserved in the large jar is unsatisfactory because of the space required for storage, the inaccessibility for

A thin section of a uterus and cervix with multiple myomas of the uterus and carcinoma of the cervix. The outer black frame is asphalt. The narrow light border is brass. The two faces of the picture are glass. The figure is reduced from 5 by 7 inches (12.7 by 17.78 cm.).

close examination, the liability to deterioration and the difficulty it offers in transportation for class or convention work.

The essential requirements for a permanent gross exhibit of pathologic change in tissue may be fully met by securing a thin section

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through the organ so as to display the largest possible cross-section area of the typical pathologic changes. By preserving this thinly cut section in natural colors in a suitable fluid contained in a thin, light cell, the requirements of small storage space, maximum teaching values, satisfactory transportation, etc., have been satisfactorily met.

This type of cell and this method of preservation have now been in use in our laboratory for more than two years, and have proved to be the most satisfactory method of exhibiting gross tissues known to us. The cells have been transported over long distances and roughly used, and have shown a very desirable permanency.

CONSTRUCTION OF CELL FOR THE MOUNTING OF A THIN SECTION OF TISSUE

Materials Required.—1. Glass plates 5 by 7 inches (12.7 by 17.78 cm.).

2. One-fourth inch strips of 15-gage soft brass, 16¾ inches (42.52 cm.) long.

3. Refined Trinidad Lake Asphalt (Genasco Brand, The Barber Asphalt Co., Philadelphia). Do not use commercial pavement asphalt.

4. Battleship linoleum, 7¼ by 4½ by ¼ inches (18.4 by 11.4 by 0.6 cm.).

5. Heavy spring paper clamps.

6. Steel spatula or putty knife.

The glass plates should be well cleansed and freed from oil by the use of sapolio or a similar substance and hot water.

The brass strips should not vary appreciably in width from end to end and should be filed or ground to an even square-cut edge. They should be cleaned and polished before the construction of the cell is begun. The strips should be cut about one third through at the points of bending, with a chisel or file. When adjusted about the linoleum mould there should be no spring exerted inward or outward.

Discoloration of the brass strips by certain tissues, such as liver, is effectually prevented by dipping the strips in duco or varnish twice, i. e., by giving them two coats of this substance.

The lump asphalt should be heated to melting several times and stirred until it is no longer frothy. Care must be exercised to prevent charring due to overheating.

Method of Assembling.—1. The bent brass strip is placed about the linoleum and adjusted to a perfect fit.

2. A plate of glass is placed on each side of the linoleum, and two spring clamps are used to hold them in position. The mould is then centered and the glasses are properly alined. Two short pieces of brass are temporarily inserted above the ends of the brass strips and held against the projecting linoleum by means of rubber bands. This will insure a uniform limitation of the asphalt to the open end of the cell and permit easy insertion of the final closing strip of brass.

3. A hot spatula is dipped into the molten asphalt and carried to the brass strip and smeared onto the glass plate, eliminating any air spaces. This procedure is followed on both glasses along one edge and finally the asphalt is filled into the full length of the groove and permitted to harden. The other two sides are treated in the same manner. It will be necessary to add some additional asphalt at the corners.

Finally the free margin of the asphalt is smoothed with a hot spatula; or the edge of the asphalt may be softened slightly with a Bunsen flame and then the edge may be pressed gently on a smooth board or strip of metal to obtain a smooth, even surface. Excess of asphalt on the glass surfaces may be removed with a knife or a razor-scraper.

4. The linoleum mould is now withdrawn, and the two short brass insertions are removed. The cell is now ready for the tissue to be inserted.

5. Fill the cell with the mounting fluid (50 per cent cane sugar saturated with thymol) to a level about 1½ inches (3.8 cm.) from the top.

6. Insert a long, thin, flat brain knife between the specimen and the glasses and liberate all imprisoned air. If the fluid becomes turbid during this operation, the turbid fluid may be displaced by inserting a long needle or cannula to the bottom of the cell and by permitting clear sugar solution to flow in by gravity.

7. Pour out excess fluid to 1½ inches (3.8 cm.) from the top and thoroughly dry the inner surfaces of the upper ends of the glasses and asphalt.

8. Insert the final strip of brass, provided with a hole bored close to one end, so that it rests on the end of the upright strips. Paste a bit of paper over the hole.

9. Fill the upper edge with asphalt, as before described.

10. With a hot needle, punch through the asphalt over the filling hole in the brass and fill the remainder of the cell by means of a hypodermic syringe and needle displacing all air.

11. Dry out the hole in the asphalt and finally sear with a hot knife point to remove all moisture and sugar.

12. Fill the hole with molten asphalt.

13. Clean the cell and finally coat the asphalt with duco to prevent stickiness.

PRESERVATION OF TISSUES ADAPTED TO THE CELL

The tissue should be fixed promptly in Pick's solution. The surface blood should be removed, preferably with physiologic solution of sodium chloride to prevent hemolysis.

Anatomic relationships should be maintained by pinning the fresh specimen to yucca board or suspending it on a glass frame. This will prevent undue warpage and distortion.

If a large specimen or an entire organ is to be fixed, such as a liver, lung or spleen, uniform fixation will be better obtained by perfusing Pick's solution through the vascular channels and suspending the organ in a large amount of the fixative.

If possible, avoid cutting the fresh specimen, because unnatural distortion due to contraction will occur, and the blood will be drained from the tissue.

If only a portion of a large organ is desired, it is best to cut a slice about 1 inch (2.5 cm.) thick and permit this to fix without distortion.

Preservation of color and proper fixation of the tissue depend, first, on maintaining the chemical concentration of the solution, and second, on the time of fixation.

All specimens should be placed in at least twice their volume of fluid. Small specimens will be well fixed in from three to five days. Large specimens, such as a spleen, breast, liver or brain, may require from five to fourteen days.

Repeated use of a given volume of fluid will deplete the chemical content and cause dilution, so that good results may not be expected.

After proper fixation, the specimen is thoroughly washed in running tap water to remove all chemicals.

The tissue section is now made, a smooth board being used, provided with two parallel 8 mm. glass rods to serve as a knife guide. A long, thin, flat blade from 14 to 16 inches (36 to 37 cm.) in length is drawn with a sweeping movement through the specimen, which is held on the cutting board between the glass rods.

The specimen is now ready to be mounted in cane sugar solution.

Formula for Pick's Fixing Solution.—Pick's solution is prepared as follows:

Chloral hydrate	50 Gm.	100 Gm.	1,000 Gm.
Carlsbad salts.....	50 Gm.	100 Gm.	1,000 Gm.
Formaldehyde	100 cc.	200 cc.	2,000 cc.
Distilled water.....	1,000 cc.	2,000 cc.	20,000 cc.

Carlsbad salts are prepared as follows:

Sodium sulphate.....	22 Gm.	220 Gm.
Sodium bicarbonate	20 Gm.	200 Gm.
Sodium chloride.....	18 Gm.	180 Gm.
Potassium nitrate.....	38 Gm.	380 Gm.
Potassium sulphate.....	2 Gm.	20 Gm.
Total	100 Gm.	1,000 Gm.

Jore's Solution.—Soft tissues, such as brain or myxomatous tumors, may be fixed in Jore's modification of Pick's solution. This has less chemical concentration and lower formaldehyde content, so that fixation is accomplished more slowly. It is prepared as follows:

Carlsbad salts.....	125 Gm.	625 Gm.
Chloral hydrate.....	125 Gm.	625 Gm.
Formaldehyde	125 cc.	625 cc.
Distilled water.....	4,000 cc.	20,000 cc.

Carlsbad salts (for 20,000 cc. of distilled water) should be made up as follows:

Sodium sulphate	137 Gm.
Sodium bicarbonate	125 Gm.
Sodium chloride	113 Gm.
Potassium nitrate	233 Gm.
Potassium sulphate	17 Gm.
Total	625 Gm.

Mounting Fluid.—Fifty per cent cane sugar in distilled water is heated to the boiling point; sufficient thymol crystals are added to saturate; the fluid is filtered through paper or porcelain, and kept well stoppered.

Do not add acid, alkaline or metallic substances to the sugar solution as a preservative, since they attack the brass. Formaldehyde should *not* be used.

A MODERN AUTOPSY TABLE

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The usual commercial autopsy table is of white porcelain, which is difficult to keep clean; it has a central drain on which the body must rest, so that, as the autopsy proceeds, there is an ever growing pool of blood and fluid around the body. H. E. Robertson [1] has devised a table consisting of a large, shallow pan, with a central drain, and a top of slats or cross-pieces which hold the body up above the pan. Through the generosity of Dr. Irving Potter and Dr. Milton Potter, a new autopsy table was recently made available for the Millard Fillmore Hospital in Buffalo. This table (figure) was constructed by the Jeffrey-Fell Company of that city, on the same principles as the one described by H. E. Robertson, but with certain improvements.

The frame of the table is of galvanized iron supported on legs of galvanized iron pipe, and this has the advantage of both strength and lightness. The height of the table can be easily changed to fit the heights of succeeding pathologists by disconnecting the lowest joint in each leg and fitting in a longer or shorter length of pipe. The frame around the top is covered by monel metal, to which is attached a shallow pan of monel metal sloping gently from the edges to a central drain which is 2 inches in diameter and 2 inches lower than the edges of the pan. This drain communicates directly with a 6 inch (15.24 cm.) sewer in the hospital and is covered by a convex cap of monel metal perforated with openings about ¼ inch (0.61 cm.) in diameter, which allows the free passage of fluids into the sewer, but holds back all tissue of appreciable size. At one side of this cap is a small curved handle by which it can be quickly and easily removed from the drain when larger pieces of tissue or blood clots are to be washed down into it.

The shallow pan is fitted with eight cross-pieces or slats made of wood and entirely covered by monel, with the seams soldered on the under side so that there are no open gaps through which infectious material may creep. These slats rest on short legs also covered by monel, and their upper surfaces are ½ inch (1.27 cm.) below the surface of the frame of the table. Of the slats, five are 5 inches (12.7 cm.) in width and three are 2½ inches (6.27 cm.) in width. The two sizes of slats are convenient in that the smaller ones offer support where comparatively little is needed, without covering up too much of the underlying pan. The larger ones are used where greater support is needed, as under the shoulders or buttocks of a heavy body, or they can be placed close together to make a cutting or examining surface on the top of the table. These eight slats seem to comprise the right number. They offer all the support needed, without covering up too much of the underlying pan. When placed tightly together they cover less than half of the table, so when they are arranged, more than half of the pan is open from above for drainage or for the washing of organs or sponges. These slats are easily sterilized either by being washed with disinfectants or by being removed entirely and placed in an autoclave.

1. Robertson, H. E.: A Post-Mortem Table (unpublished communication), read before the International Association of Medical Museums, March, 1929, Chicago.

Above this table is the pipe for the delivery of water to the table, and to this is attached a suction pump. At the head of the table is a clamp holding a head-rest, which can be quickly removed or adjusted to any height, and turned either over the table or projecting out beyond the end of it.

This autopsy table has many advantages. The monel metal does not stain or discolor as easily as white, and yet, unlike zinc or iron, which turns dark and appears to be dirty, it always looks clean and bright, and a little rubbing makes it shine like a silver plate. There is no part of the table that can harbor infectious material, and it is easily sterilized. It is light and easily movable except for the attachment of the central drain. It is long enough to allow at the foot a wooden stand for the cutting of organs or for the placing of extra instruments or solutions. It is wide enough to accommodate a large body, or if necessary, two bodies, and to allow many people to work around it. There is free drainage from any one part of

Autopsy table with head-rest and slats in place ready for use.

the body into the underlying pan, without soiling or contaminating any other part of the body. For example, pus from an abscess will drain directly down into the pan without running over or about the body. The adjustable cross-pieces give sufficient support in the right places for either an infant or an obese man. Removed organs may be dropped into the underlying pan, out of the way, where they will be automatically washed by the running water until they are needed or discarded. Also, the pan, being lower than the surface of the table, prevents the usual splashing or spraying of fine particles of fluid, which may or may not be infectious, from the surface of the table. This is of real value in preventing contamination of the clothing of the spectators, who may go from the autopsy room to other parts of the hospital or to visit patients. Also, on this table, an autopsy can be made with the minimum amount of soiling or discoloration of the body by blood or fluids, and consequently it will offer much less offense to the spectators and will be a factor in destroying an aversion to autopsies, which will help to obtain more autopsies.

General Review

EXPERIMENTAL STUDY OF TRAUMATIC SHOCK

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The present review was undertaken to ascertain, primarily, the trend of experimental investigation on the ever current subject of traumatic shock. Mann in 1914 came to the somewhat discouraging conclusion that "despite the enormous amount of work which has been done on surgical shock there is still among recent writers no general agreement as to the causes and nature of this condition. A critical review of the literature reveals an astounding amount of contradictory experimental data, and a great number of diverse conclusions based thereon." He found no common standard of shock. "In protocols of some observers a markedly lowered blood pressure was taken as the sole indication of the presence of shock. In others it seemed to have been thought sufficient to state—'after reducing the animal to a state of shock,' without giving the methods of its production or any criteria by which it is possible to judge whether an animal really was or was not in a condition of shock. This lack of definiteness in regard to these fundamental matters made many of the most extensive researches on shock of somewhat questionable value." Cope in 1928 was dissatisfied with popular conceptions of shock and found eight textbook definitions of it inadequate or ambiguous. Some of them obviously were definitions of collapse; some were couched in such terms as to make shock and collapse synonymous. Cope deplored the fact that most of these definitions were based on a consideration of traumatic shock alone and argued for the adoption of a broad point of view—"just as fever is so considered. Shock is a depression, as fever is generally an exaltation, of the phenomena of vital activity." He maintained, ". . . the term shock signifies a condition following the application of harmful stimuli (traumatic or toxic) or the depletion of the body fluids, in which there is clinically demonstrable depression of the vital processes of the body, particularly the circulation and metabolism. Collapse is the term applicable to the sudden onset or rapid aggravation of the symptoms of

From the Norman Bridge Pathological Laboratory of Rush Medical College of the University of Chicago.

shock." Mann favored a definition that does not involve any more or less questionable theory of etiology, such as that suggested by Meltzer. "Shock is a state of general apathy, reduced sensibility, extreme motor weakness, great pallor, very rapid, small pulse, thready, soft arteries, irregular gasping respirations and subnormal temperature."

Confusion in delimiting shock is reflected in the numerous theories of its origin. Discussion of these theories and details of their individual merits are abundant in the voluminous literature on traumatic shock (Mann, Cope, Risley, Macleod, Cowell, Parsons and Phemister). The favorite procedure in many publications, of a preliminary discussion of theories, has been justified by the astounding discrepancies between theory and fact later disclosed by huge series of well controlled experiments on animals, laboratory tests and clinical observations. The chaotic impression created by an ensemble of the results of investigations on shock is lacking in many individual researches which are replete with evidence of ingenuity, resource and logical deduction. Gradually a more accurate appraisal of the phenomena of shock has resulted in a grouping into essential and auxiliary features. Simplification of the concept of shock has brought into prominence the basic character of alterations in the volume of the blood in the production of this condition. Proof of the importance of this factor in the production of shock is intimately coupled with disproof of the importance of other factors. In tracing the evolution of modern notions of shock, I have found it helpful to resort to somewhat detailed experimental evidence for and against the various theories.

THE VASOMOTOR EXHAUSTION THEORY

Because of the marked physical and mental depression occurring in shock, exhaustion of vital nerve centers was held responsible for the condition. Painful stimuli arising from a wound were thought to produce the exhaustion. Crile (1923 [*b*]; 1922) has been the most ardent exponent of this line of reasoning and has demonstrated morphologic and conduction changes in the tissues of rabbits following exhaustion produced in various ways. He pointed out that all cells are composed of two colloids separated from each other and from the outside by nuclear and cell membranes. The nuclear material is acid in nature; the cell body, alkaline. The nucleus of a cell as compared with the cytoplasm is positive. Each cell is therefore an ultimate electrochemical unit, and each organ and tissue an electrochemical mechanism. The normal conditions in the body obtain when the brain acts as the positive pole and the liver as the negative pole, and when adequate oxygen and epinephrine are available. On the basis of conduction measurements he assumed that the first effect of stimulation within the organism is

a slight decrease in the conductivity of the cerebrum followed by a gradual continuous decrease. The results indicate that the intracellular changes in excitation and exhaustion that are revealed by the microscope are paralleled by alterations in electrical conductivity, and that both the histologic and the electrical changes bear a direct relation to the vitality of the organ. When differential stainability of the nerve cells is lost, these cells have ceased to be efficient batteries.

Dolley described morphologic changes in nerve cells in shock and severe anemia and found these changes identical in the two conditions. He was of the opinion that the cerebral cells had no chance or means of recuperation and, driven by never ceasing stimulation, ran their life cycle in a few hours. A definite sequence of alterations in the relation between the extranuclear and intranuclear chromatin material was believed to represent functional activity carried to its ultimate limit. The essence of the process, so he said, was the continued overproduction of chromatic material up to the limit of capacity, followed by using it up from this maximum point down to its complete disappearance from cell body and nucleus. He found that these alterations were most clearly evident in the Purkinje cells of the cerebellum and were observed in three stages. In the first there was a disappearance of extranuclear chromatic material; in the second, a using up of diffused intranuclear chromatic material after it passes in turn into the cytoplasm, leaving the karyosome intact, and, in the third, the giving up of the chromatic material by the karyosome which, after diffusion into the cytoplasm, also died. By means of camera lucida drawings, cell volume and nucleus-plasma ratios were accurately determined.

Confirmation of these cellular changes in the central nervous system is supplied, in some measure, by Mott, who studied the brains of soldiers dead from shell shock, with and without burial and with probable gas poisoning; the brains of three men dying of wound shock; the brain of a person who died of shock following extensive burns; brains removed after death from anaphylactic shock; the brain of one who died of shock following contusion of the heart; the brain of a man who died under anesthesia, and brains of animals which had been given injections of histamine. The material acquired from such varied sources had two classes of alterations in common, chromatolytic and biochemical. Routine sections were made of medulla in order to study the vital centers, and of cerebellum because of its uniform structure. In the brains of bodies dead from shock there was a definite diminution of basophilic substance and a diffuse purple-staining of the cells.

Opposed to these views that exhaustion is an initiating factor in shock are others which indicate that it is of relatively little importance. Janeway and Ewing were unable to produce shock by afferent electrical stimulation for two hours in the presence of a good blood pressure

and unimpaired vasomotor compensatory mechanism. Mann performed two crucial experiments to show that shock produced by the easiest and most certain method, traumatization and exposure of the abdominal viscera, is not due alone to paralysis of the vasomotor mechanism of the splanchnic area. By section of the spinal cord or of the splanchnic nerves he was not able to produce shock. By removing the thoracic and abdominal organs from an animal, the "visceral organism" could still be kept alive many hours, and function. Therefore he concluded that some cause other than vasomotor paralysis or inhibition was involved in the production of shock. Cannon (1922) transected the spinal cord above the lumbar plexus and severed all the nerves to a cat's hind limb before traumatizing it with a hammer, but was unable by these precautions to prevent the development of shock. In his experiments he found no evidence that impulses caused by trauma might produce reflex inhibition of the vasomotor center.

Porter, working on cats, found that stimulation of the depressor nerve caused a 46 per cent fall in blood pressure. After this animal lay in shock for eight hours, with exposed intestines painted with nitric acid, stimulation of the depressor nerve still caused a 45 per cent fall in blood pressure. In order to test the effect of frequently repeated stimuli over long periods, the depressor nerve of a cat was electrically stimulated at intervals for eight hours. The initial stimulation, with a current of 1,000 Kronecker units, produced a fall equal to 35 mm. of mercury. A final stimulus of the same intensity caused a fall of 42 mm. Other experiments on cats near death from pneumonia and on a rabbit immediately after it had died of diphtheria proved the normal reactivity of the vasomotor center. Porter pointed out that since the depressor nerve can affect the blood pressure only through the vasomotor center, the vasomotor center is not exhausted in shock. He concluded:

> Experimental evidence shows that failure of the vasomotor center is due to anemia rather than stimulation. If the blood pressure in the nutrient capillaries falls below the critical nutrient level—a very low level—the vasomotor cells are at once affected. Bulbar anemia may be a consequence of prolonged shock especially when the bulb is allowed to be higher than the trunk, for a man with shock is a gravity animal and his cerebral circulation then depends chiefly on small differences in hydrostatic level, but an injury after shock is not to be confused with an injury causing shock. In short, exhaustion of the vasomotor center is a taking phrase but it is nothing more.

THE FAT EMBOLISM THEORY

In 1916, Porter was informed by the French military surgeons at the Carrel Hospital in Compiègne that shock occurred most frequently after shell fracture of the femur and after multiple wounds through

the subcutaneous fat. He verified this statement soon afterward from observations on over one thousand freshly wounded soldiers, and then proceeded to prove experimentally that fat embolism might cause shock. Curiously he found that "notwithstanding the very numerous clinical and pathological studies of fat embolism there has been, heretofore, no attempt made to demonstrate by measurement of the blood pressure a causal relation between fat embolism and the shock of the battlefield." By injecting fatty substances such as olive oil and thick cream into the jugular veins of cats he was able to reproduce in them the clinical picture of shock. In order to exclude embolism of the lungs and heart, Porter curarized a rabbit, instituted artificial respiration, ligated the subclavian artery just after its exit from the aorta and distal to the vertebral artery and tied off all but two muscular branches. With the vertebral and two muscle arteries the only ones patent, he then injected 0.1 cc. of oil per kilogram of body weight. In fifteen minutes, the carotid diastolic blood pressure had fallen from 160 mm. of mercury to 20. Fat was hard to find in sections of the vasomotor center stained with scharlach r when such small quantities of oil were employed. The physiologic evidence in favor of fat embolism is, however, convincing because all portions of the brain in a cat may be excised without lowering the blood pressure so long as the vasomotor center is left intact. Thus Porter attributed the fall in blood pressure after fat embolism to injury of this center. As some experimenters had been unable to produce a fall in blood pressure by injecting fat into the peripheral end of the carotid artery, they concluded that the important features of fat embolism were associated with embolism of the lungs rather than the brain. Clinging steadfastly to this theory of fat embolism of the vasomotor center, Porter pointed out that the vasomotor center derives its blood supply from the basilar artery and not from the carotids. He recognized the rich anastomoses between branches of these vessels in the circle of Willis, but believed that this circle is a balanced pressure ring in which pressure from each carotid region contends with that from the basilar region. Thus oil injected into the carotid arteries seldom caused shock because it entered parts of the brain anterior to the bulb. If, however, one carotid artery was clamped and oil injected into the other, shock usually occurred. When both carotid arteries were tied, the vertebrals left patent and oil injected into the external jugular vein of a cat, shock usually took place. When, on the other hand, the vertebral arteries were tied and both carotids were free and oil was injected into the external jugular vein, shock seldom occurred.

Under the conditions of the last two experiments, the lungs were embolized equally in both series, but since the results were decidedly different, pulmonary embolism could not have been of great importance.

The interpretation of these results by Porter was again embolization of the vasomotor center via the vertebral arteries.

Mott in his studies of brains from soldiers who died in shock found fat emboli in the medulla of two who had shell-fractured femurs, but in no others was fat evident. This corroborative evidence of Porter's fat embolism theory is, however, exceedingly scanty as compared with the numerous objections to it raised on theoretical, clinical and experimental grounds. Wieting in his extensive review of the literature ascribed no importance to either fat or air embolism in the causation of shock. Cannon (1922) found the fat embolism theory defective in that it offers no adequate explanation for the onset of delayed or secondary shock. It also fails to account for the occurrence, both in clinical and experimental shock, of a diminution of blood volume and either a local or a general concentration of corpuscles. Parsons and Phemister found fat in the lungs of each of ten dogs the limbs of which had been hammered. In several this was slight; in three, moderate. The central nervous systems of four dogs were examined for fat after trauma, and in three of the dogs, bones were intentionally broken to favor the development of fat embolism. There was practically no fat in the blood vessels of the cerebrum, cerebellum or medulla, and so these authors concluded that fat was too scanty to have played a rôle in the production of shock.

THE ACAPNIA THEORY

The acapnia theory of Y. Henderson (1908) was regarded as promising before attempts were made to ascertain the relative importance of the different alterations that, taken altogether, constitute shock. According to Henderson, exhalation of carbon dioxide is responsible for the development of apnea and eventually respiratory failure. This exhalation may result clinically from a violent and prolonged hyperpnea caused by stimulation of afferent nerves during injury. It may also occur when the abdomen is opened in surgical operations, even when cooling and drying are prevented. The marked hyperemia of the viscera thus exposed, even when no further procedures are undertaken, is presumably due to loss of carbon dioxide and is relieved by placing the part in saline solution saturated with carbon dioxide. Experimental hyperventilation of the lungs may result in a shocklike condition. The theory acquired support from studies on gas tension in the blood and tissues, especially studies of mountain sickness. In this connection, Henderson cited the work of the Italian investigators, Mosso and Marro, who found by blood gas analyses that when animals were transported up to the physiologic laboratory on Monte Rosa or placed in the low pressure chamber at Turin, the carbon dioxide content of their arterial blood was reduced. Unlimited quantities of pure oxygen

were incapable of averting muscular weakness, sleepiness and, at times, almost vomiting in animals subjected to decreased barometric pressure.

This attractive, interesting hypothesis of Henderson's was soon confronted by the results of experiments by a number of investigators. Mann prevented escape of carbon dioxide from the abdomen of the dog by making a small incision, inserting his gloved hand and then clamping the skin of the animal tightly about his wrist before traumatizing the viscera. In other experiments he passed a stream of gas containing a high percentage of carbon dioxide into the abdomen. Despite these precautions, the animals went into shock. Janeway and Ewing instituted artificial hyperventilation in dogs up to seventy breaths a minute, produced a fall of up to 50 per cent of the carbon dioxide of the blood in one-half hour and had the animals in shock in from two to three hours. The dogs had interstitial emphysema of the lungs caused by the excessive pressure necessary for this type of experiment. Still more subversive for the acapnia theory were their experiments in which they provided a rebreathing apparatus for carbon dioxide or kept the pressure constant from a tank of this gas and achieved results identical with those in which such provisions were lacking. They were thus forced to disregard diminution in carbon dioxide as the cause of shock. By employing simultaneously a cardiometer in circuit with a recording tambour, a water manometer connected with a bronchus and a blood pressure cannula in the carotid artery, they proved that increased intrathoracic pressure was the all-important factor under the condition of hyperventilation. This increased intrathoracic pressure hindered venous return to the heart by direct pressure around the pulmonary artery and on the capillaries and veins within the lungs themselves. In an experiment, an increase of intrabronchial pressure from 8 to 30 mm. of mercury caused a drop in blood pressure from 122 to 55 mm. of mercury and a 44 per cent diminution of cardiac output. A rise of intrabronchial pressure above 8 or 10 mm. of mercury always caused a fall of blood pressure proportional to the rise of intrabronchial pressure. As an improvement over previous investigations on abdominal ventilation, these men sewed a celluloid window between the recti muscles so that they could observe the viscera and control aeration with warm, moist air. When intestinal peristalsis was almost absent, the blood pressure was still 163 mm. of mercury. After removal of this window and aeration for forty-five minutes longer, the blood pressure was 153 mm. of mercury and the carbon dioxide of the arterial blood 38.8 per cent by volume. After the intestines had been handled for ten minutes, the blood pressure was 80 mm., and after twenty minutes, 56 mm. of mercury. At the end of thirty minutes, the carbon dioxide was 31.6 per cent by volume. Still other experiments were performed in which the carbon dioxide in the blood was kept at a normal level

through a long tube inserted into the trachea. After aeration of the intestines for one and one-half hours with the abdominal wall and omentum removed, the blood pressure remained unchanged and the animal was in good condition. At the end of this time, the intestines were handled, and in ten minutes the blood pressure fell from 122 to 60 mm. of mercury, although the carbon dioxide content of the blood was 45.1 per cent by volume. In twenty-five minutes, the blood pressure was 46 mm. of mercury, the carbon dioxide was undiminished, and the dog was in profound shock. Stimulation of the sciatic nerve at this time caused the blood pressure to rise to 96 mm. of mercury, indicating a strong medullary reaction.

THE ACIDOSIS THEORY

The carbon dioxide content of the blood in shock has also been studied thoroughly from another angle—its possible relationship to acidosis as the cause of shock. Controversy on this point soon subsided when investigators qualified the term of acidosis in the sense of L. T. Henderson and Van Slyke—a diminished alkali reserve rather than a marked change in hydrogen ion concentration. Studies of the alkali reserve have been made as a routine by many experimenters, regardless of what features of shock they were concerned with or to what theory they subscribed. The result has been a concurrence of opinion, an all too rare situation on almost any phase of traumatic shock. The researches of the British Shock Commission helped to establish the precise relationship of acidosis to shock. The first of a series of experiments consisted in injecting 30 cc. of normal lactic acid into the internal saphenous vein of a cat weighing 3 Kg. in a period of twenty-four minutes. Before the infusion, the combined carbon dioxide content of plasma removed from the jugular vein was 46.4 per cent by volume; during the last minute of infusion, it was 10.3; ten minutes later, 22.25, and one hour later, 32.1.

The animal was not in shock after intravenous injection of an amount of acid far in excess of any that would be formed in its muscles after fatigue or injury. Since acidosis produced in this manner was more simple than that which might occur in shock following wounds, acidosis was combined with hemorrhage, with injections of histamine, epinephrine hydrochloride and peptone and with tests of activity of the vasomotor center. In no instance did acidosis modify the action of the other agents in reducing the carbon dioxide-combining power of the blood. It was found that hemorrhage severe enough to reduce the blood pressure to 79 mm. of mercury caused rapid and serious decline of the alkali reserve. A cannula tied into the pericardial sac and connected to a buret filled with physiologic solution of sodium

chloride served as a variable system for restricting the filling of the heart. By simply raising or lowering the reservoir, pressure within the sac could be maintained at any desired level, and so various blood pressures could be affected. When a blood pressure of 80 mm. of mercury was maintained in the animal's arteries, no ill effects were noted, but when this was forced down to 60, acidosis developed. If, however, 20 per cent of the animal's blood was removed by bleeding, a reduction of its blood pressure to 80 mm. was sufficient to cause acidosis. The normal average alkali reserve of the dog's venous plasma is 43.4 per cent by volume, with variations ranging from 32.4 to 59.5. Raymond found that the mean alkali reserve fell to 26.1 if ether anesthesia was maintained for from forty-five minutes to two hours, but that the mean fall decreased per unit of time after this period. In some dogs, the alkali reserve fell no lower in profound shock than in experiments of similar duration under ether anesthesia control performed on the same animals a week previously, from which they completely recovered. He could not discover a critical level of alkali reserve. Cannon and Cattell, using an intrapericardial cannula, were convinced that the critical level in blood pressure, below which the alkali reserve diminishes, is 80 mm. of mercury. The unanimity of opinion as to the occurrence of a diminution of alkali reserve in shock is not evident in the explanations offered for its development. The oldest view that a nonrespirable acid such as lactic acid develops in the tissues because of a want of oxygen, unites with the sodium of the sodium bicarbonate and then drives off the carbon dioxide, which is breathed out, was termed by Cannon the acidotic theory. Y. Henderson saw in this diminished alkali reserve support for his acapnial theory.

The process viewed in this light was based on the assumption that excessive respiration diminishes the carbonic acid in the blood with consequent alkalosis, and that thereupon the extra alkali disappears into the tissues or diffuses into the body fluids outside the blood or escapes through the kidneys. An explanation, based on experience with gas bacillus infection, was offered by Wright. He proved that in its growth B. welchii diminishes the antitryptic power of the medium and renders it acid. These chemical changes furnish the conditions required for the avalanchelike inroad of the bacterial infection. Experiments by the Medical Research Committee proved that it is the toxemia of the gas gangrene type that produces the acidosis rather than a change in reaction in the tissue fluids. In a specific experiment, 20 cc. of alkaline fluid from the edematous muscles of a puppy infected with the vibrion septique was injected into a vein of another puppy during a period of twenty-five minutes. Although the fluid that had been injected was alkaline, fifteen minutes after the injection the puppy's blood pressure had fallen from 116 mm. of mercury to 64 and the alkali

reserve from 43 to 27 per cent by volume. Half an hour later the blood pressure was 30 and the alkali reserve 7. Cannon is supported by several investigators (McEllroy, Erlanger and Gasser, Gesell, Penfield) in his contention that all conditions associated with an increase of lactic acid or a reduced alkali reserve are characterized by diminished blood volume or diminished blood pressure or decreased carrying power of the corpuscles, so that delivery of oxygen is reduced.

B. Moore also tried to combine the various views on diminished alkali reserve on an acapnial basis. He assumed that as a consequence of shock there is a general lowering of metabolic activity to about one third of the normal rate. If then the lungs continue to function at their usual rate, or even less, there will be an excess of carbon dioxide removed from the blood, and the blood will become more alkaline. Then the kidneys and tissue cells will remove alkali from the circulation, and the blood then examined will have less sodium bicarbonate in it than normally. The excessive breathing held necessary by Henderson and Haggard for the development of acidosis is, according to Cannon (1922), not present in wounded soldiers, whose respirations in shock are shallow, feeble and interrupted by occasional sighs.

Aub studied the basal metabolism of cats under ethyl carbamate anesthesia, and found an increase in pulmonary ventilation of 54 per cent after the hind limbs had been crushed, but before the onset of shock. He found that the basal metabolism decreased 19 per cent after mild shock and 30 per cent after severe shock. Increase of intrapericardial pressure to reduce the blood pressure to a critical level of 75 or 80 mm. of mercury caused an average decrease in metabolism of 31 per cent. The prompt reduction of metabolism by this method of merely hindering the venous return to the heart is evidence that some mechanical factor such as retarded blood flow is the cause of reduced metabolism. Aub was certain, however, that reduced blood pressure alone does not account for the fall of metabolism, because a low blood pressure following hemorrhage may be associated with only a slight drop in metabolism. After muscle injury, the metabolism may be reduced before a great fall in blood pressure has occurred. A possible explanation of these discrepancies is offered by Gesell, who found that in the early stages of shock produced by injury of tissue there is usually a marked reduction in the volume flow of blood in the peripheral organs, although the blood pressure is only a little changed. The volume flow of blood determines the delivery of oxygen to the tissues, and this may vary to some degree without a corresponding variation in the blood pressure.

THE TOXEMIA THEORY

The resemblance of a patient in traumatic shock to one prostrated from some overwhelming toxemia has been stressed repeatedly and

investigated extensively. In certain patients, e. g., those with gas bacillus infections, the connection between toxemia and shock was evident. In the great majority of persons suffering from shock during the war this condition developed before they could have been infected with gas-forming organisms. The thought of such an infection is not tenable in the case of civilians or that of laboratory animals. Some common factor producing the symptoms of shock in animals and men after injury was demanded. One result of efforts to answer this requirement properly has been the attention directed to the refuse of damaged tissues. The proteins and their cleavage products were suspected of exerting such an influence when abnormally liberated in the body, because of numerous physiologic researches with peptone. Vincent and Sheen, using saline decoctions and proteid, alcoholic, and ether extracts intravenously in cats, dogs and rabbits, noted a marked depressor effect of such extracts. Toxic substances were found in the fluid in a closed loop of bowel by Whipple, Stone and Bernheim, who were concerned with intestinal obstruction. The toxic element of fluid from a closed intestinal loop was found to emanate in the mucosa. The crowning achievement in production of shock by the injection of toxic agents was the use of histamine by Dale and Laidlaw. Shock produced by this substance could be varied according to the amount employed and the time allowed for its administration. In guinea-pigs and rabbits, small doses produced phenomena usually associated with anaphylactic shock. Sudden introduction of a large dose resulted in effects divisible into three phases: The first was marked by an immediate fall in blood pressure of about 60 mm. of mercury, due to constriction of the pulmonary arterial branches and a diminished output of the left ventricle of the heart. The second phase, which, like the first, lasted only a few seconds, was marked by an arrest of the downward tendency of the arterial pressure and by a secondary rise due to the stimulating action of the drug causing vasoconstriction and generalized contraction of smooth muscle. Finally, about four minutes after injection of this poison, symptoms of shock set in.

To approximate more nearly conditions in which shock is seen clinically, Cannon and Bayliss bruised the muscles of a cat's thigh and noted a prompt fall in blood pressure. This fall failed to appear if the aorta was clamped above the bifurcation during the trauma, but was not averted by transsection of the spinal cord in the lumbar region. The fall, therefore, was presumably due to a combination of dilation of blood vessels in the injured area, together with some extravasation of blood, and the rapid setting free of a chemical substance acting as a powerful depressant on the blood pressure. The secondary fall in pressure was explained by the continued absorption of the depressant substance and by the establishment of a vicious circle in consequence

of the low blood pressure. Subsequently Cannon (1922), by elaboration of this experiment, sought to establish a connection between local injury of tissue and the general bodily condition through the circulation. Usually when the muscles of the hind leg of a cat were crushed, shock developed in about twenty minutes. When, however, the femoral artery and vein were ligated prior to trauma, shock did not develop in even thirty-three minutes. When the ligatures were removed from the vessels there ensued a marked fall in blood pressure, which diminished after the vessels had again been ligated. When a constant level in blood pressure had been reached, massage of the crushed muscle produced a further drop in blood pressure, a discovery that had a practical bearing on the immobilization of bony fragments. Whatever the nature of the substance in the damaged tissues, it seemed to be fairly promptly changed in the body or eliminated.

Obviously, most of the evidence for a toxic cause of traumatic shock obtained from experiments has been based on the use of histamine or on the assumption that a histamine-like substance is formed in the injured tissues. Histamine has been isolated from the dog's intestinal mucosa (Dale). Search for other sources of toxic material has also borne fruit. A toxin seems to be liberated by the breakdown of platelets from the blood of horses, rabbits and dogs. Greeley obtained quantities of this toxin sufficient to kill the animal from a number of platelets that it is easily capable of providing. He stated his belief that trauma to the labile platelets during accidents and operations may result in their disintegration. He was not in accord with von Behring, who found clumped platelets in the cerebral vessels in anaphylactic shock and attributed the phenomenon of such shock to their presence. Dale and Laidlaw found large clumps of agglutinated platelets in films of blood from animals in histamine shock. Greeley guarded against thrombosis in some of his animals by repeatedly defibrinating large amounts of their blood and then reinjecting it and also rendered the blood incoagulable by injections of heparin. His platelet extract under such circumstances was still capable of producing shock and death.

Extensive experience with wounded men in shock led many surgeons to suspect a toxic factor in the development of shock. Quenu summarized the observations of French surgeons on this subject in the following way: Secondary or traumatic shock does not appear immediately after the receipt of wounds, and therefore is not due to a nervous effect. Shock is frequently well established before infection has set in; therefore it is not of bacterial origin. Shock is most frequently seen after extensive damage of muscles or after multiple wounds. Support for this view was given Quenu by the Medical Research Committee after determining that there was an increase in undeter-

mined nitrogen, total nonprotein nitrogen and residual nitrogen in the blood of patients in shock. This was accounted for as a consequence of the absorption of material from the traumatized area and also as perhaps due to the effect of damage done to tissue by circulating toxins. Recently studies of metabolism following reduction of high blood pressure have suggested a much simpler explanation for these disturbances in body chemistry and will be referred to later. Quenu noted also that factors favoring absorption at the region of injury favored the production of shock. Thus shock was most severe when the region of damage communicated with the exterior by only a small orifice. The negative aspect of this evidence was presented by soldiers in whom a large fleshy mass, together with the skin that covered it, was carried away by a missile; in these soldiers very slight shock developed, or none. Anything that delayed or checked absorption from the injured region delayed the development of shock, but if there was a sudden removal of this check serious results followed. Thus war surgeons saw shock develop rapidly after removal of a tourniquet from a crushed extremity or after the clearing of timbers and débris from a pinioned limb.

Suppression of the injured area, if not too long delayed, caused shock to disappear. Thus McKee and Wallace and their co-workers noted the marked improvement that followed removal of a mangled limb and were able to keep the mortality down to 11 per cent if operations were performed within three hours of the injury. Amputations performed eight hours after injury were attended by 75 per cent mortality.

Attempts to cross the circulation of an animal in shock with that of a normal animal have been made with various results. Crile (1923 [*a*]) was unable to produce shock in a normal dog in which the carotid artery and the internal jugular vein were anastomosed to a dog in which shock was produced by intestinal manipulation. Bazett and Quinby managed to cross the entire systemic circulation from one animal to another by means of an anastomosis made at the level of the ascending part of the aorta. In experiments on shock, they found that the animal in better condition bled into the other, so that no definite evidence could be obtained. Janeway and Ewing also found that their experiments were unsatisfactory because the recipient drained off so much blood that the donor became exsanguinated. McIver and Haggart avoided the difficulties experienced by previous investigators by crossing only the circulation from the traumatized lower extremities of one cat to the intact cephalic half of another cat. The caudal half of this second cat was amputated to prevent the flow of blood through the large veins in the spinal canal. With this method shock developed

in nine of the twelve cats that had taken over the circulation from the traumatized region. In the three instances in which shock did not develop, the experiments were discontinued at the end of one and one-half hours.

Quenu stated that blood from an animal in shock will, on intravascular injection, cause shock in a second animal. Parsons and Phemister found alcoholic extracts of blood extravasated into traumatized limbs produced, on intravenous injection, a fall of blood pressure no greater than that produced by an extract of the same amount of normal blood. They investigated various possible toxic elements of shock in forty-five dogs the extremities of which were traumatized, but with consistently negative results. Thus they found only minute traces of hemoglobin in the plasma of blood drawn from the jugular vein. This was evidence against the view that damaged, extravasated or hemolyzed blood in the traumatized limb might enter the circulation and bring about a fall in blood pressure. Blood from the femoral vein of a traumatized limb was collected, without shaking, in a viviperfusion flask and then circulated through the femoral artery of a normal dog without producing vasodilation in the limb or a fall in general blood pressure. In order to determine the presence of toxic substances in devitalized muscle, the entire right rectus muscle was excised aseptically and reimplanted in three dogs, intraperitoneally in two, and into its bed in one. The animals were observed for three weeks after operation, but showed no signs of intoxication.

Because of the many experiments in which shock was produced by intestinal trauma and in which resulting visceral hyperemia ensued, the idea of splanchnic engorgement in shock produced in other ways was prevalent. Wallace and other surgeons were convinced, however, that splanchnic engorgement is not present in shock, after they had performed many hundred laparotomies on wounded soldiers. R. M. Moore determined the state of contraction of the spleen in various stages of traumatic shock, and in every instance found the spleen so extremely constricted that surfaces made by cutting did not bleed. The spleens were found to average about 0.25 per cent of the body weight—a weight that corresponds to the minimum for a completely contracted spleen. Moore regarded the spleen as a large and modified splanchnic blood vessel, the volume of which serves as an index of the state of contraction of the splanchnic vascular bed as a whole.

The occurrence of extreme contraction of the spleen in traumatic shock is interpreted as supporting the observations of other investigators to the effect that the splanchnic vasomotor mechanism becomes highly active in shock in an attempt to maintain an effective blood pressure in the presence of rapidly diminishing blood volume.

CAPILLARY CHANGES IN TRAUMATIC SHOCK

With the development of newer ideas on capillary circulation the importance of the capillary stasis of blood in traumatic shock was emphasized. The direct observation of capillaries by such investigators as Krogh, Landis, Densham and Wells, Hooker, and Hill and McQueen revealed the potential vastness of the capillary bed. It is now generally conceded that not all capillaries are actively engaged in circulatory activities at all times. It was assumed by some of the earlier observers that those capillaries not filled with blood were too small to admit blood cells, but served only for the transport of plasma. More recently the interpretation applied to these closed vessels is that they represent the reserve irrigating channels that are opened only when the muscle is actively contracting. In muscles at rest there is a definite regularity in the distribution of patent capillaries, just sufficient channels participating in the circulation to satisfy the metabolic requirements. The distance between capillaries apparently is almost constant not only for resting and active stages in general, but also for a given group of muscles. Thus the capillaries in a frog's rectus abdominis muscle are spaced at intervals of 60 microns, whereas in most of the muscles of the trunk the open capillaries are from 200 to 500 microns apart. Permanent preparations of resting and active muscle have been made by Krogh after the capillaries had received an injection of india ink. Another observation made by him was that frog's corpuscles frequently pass through capillaries smaller than the smallest dimension of the corpuscle by a process of lateral folding or longitudinal stretching. When the muscles are active, their capillaries dilate and allow freer passage of corpuscles.

The oxygen pressure in muscles at rest is very low, but in working muscles it approaches very near to that of the blood (Densham and Wells). Although the normal tension of oxygen in the capillaries is small, insufficient oxygen for even short intervals has disastrous effects. Immediately after a three-minute period of oxygen lack, fluid filtered through the capillary wall at approximately four times the normal rate. This increased permeability of the wall also permitted the passage of protein, reducing the effective osmotic pressure of the plasma proteins to almost one-half their normal value. Exposure of the frog's mesentery to Ringer's solution, one-half saturated with carbon dioxide, produced no change in capillary fluid movement, while complete saturation increased the rate of fluid movement very slightly, but the wall remained normally impermeable to protein.

Increase of hydrogen ion concentration produced almost no change of fluid movement within physiologic limits, though at p_H 4 the characteristic effects of injury occurred. Hill and McQueen noted the

existence of direct anastomotic vessels between capillaries and venules. If no such vessels existed, they maintained, the slowing in the blood current in the capillary area would uniformly lead to concentration so great that little or no blood would be in the circulation. Normally, differences greater than 3 per cent do not exist between venous and capillary erythrocyte counts. This is assumed from the usual conception of the circulation as passing from the arterioles through the capillaries into venules and then into veins. In traumatic shock in men there are not infrequently 2,000,000 fewer red blood cells per cubic millimeter in the venous blood than in that from capillaries. The anastomotic channels would, under such circumstances, maintain a certain minimum amount of blood in circulation. When eventually capillary stasis in shock reduces the available circulation through these channels, disaster results.

Cannon, Frazer and Hooper noted marked capillary stagnation and high red cell counts in severe shock and found that this condition once established was only gradually recovered from. Restoration to normal venous and capillary blood cell ratios often required three days. These observations were evidence in favor of the view that the circulatory difficulty in shock is due to loss of blood from the circulation, though not from the body. This withholding of blood from the normal currency explained most easily the existence of shock in men who had not bled. Such a conception of shock was a radical departure from the original one, and Cannon suggested the hippocratic term "exaemia" —drained of blood—as being more descriptive. Cannon is inclined to agree with Cowell that primary shock, coming on immediately after a wound, is best accounted for as the result of nervous action. Fainting after slight wounds is a transient state of this nature, which in true shock is more persistent. A combination of facts to explain secondary shock and stress exemia was accomplished in the following fashion: Sweating and exposure lead to rapid loss of heat from the body, and low external temperature favors the process. Inactivity of the wounded man and absence of shivering lessens production of heat. Thus the body becomes cold. In consequence of the low blood pressure aided by chilled tissues, there is stagnation of corpuscles in the capillaries. The onward flow here checked undergoes concentration, so that the capillary red cell count is high. Prolonged lack of fluid and sweating may favor the stagnation and further concentration of the blood. By accumulation in the capillaries the return of blood to the heart is lessened until a persistent low blood pressure becomes established. The blood lost from currency produces a state equivalent to hemorrhage. Any true hemorrhage therefore exaggerates the existence of shock or exemia.

THE RELATION BETWEEN HEMORRHAGE AND SHOCK

The close connection between hemorrhage and shock has been stressed repeatedly (Mann, Parsons and Phemister). Mann was unable to differentiate between shock and the results of hemorrhage "except perhaps by a leucocyte count." Cannon, Frazer and Hooper have stressed the importance of hemorrhage in hastening the onset of shock. Blalock and his associates are convinced that loss of blood plasma is the important event that precedes the onset of shock. Their original experiments were undertaken to determine the oxygen content of the blood in shock, since lack of oxygen, as a result of decreased volume of blood, was credited with harmful effects on the tissues. Samples of blood from the right auricle, the renal, portal, external jugular and femoral veins and the femoral artery were tested for oxygen after shock had been produced in dogs by hemorrhage, injections of histamine and trauma to the cerebrum, intestines or one leg. Approximately the same relationship existed between the values of the oxygen content of the blood from various sites after low blood pressure had been produced by hemorrhage, histamine and trauma to the brain. The oxygen content of the blood from the portal vein was much higher relatively after trauma to the intestine, and that of the blood from the femoral vein of a traumatized leg was higher than that of the opposite extremity. The observations suggested a local accumulation of blood at the site of injury to a large region such as the intestine or an extremity and were evidence against the action of a histamine-like substance that produced a general bodily effect. Henderson, working with shock induced by intestinal trauma in dogs, found the oxygen content of the venous blood decreased. He attributed the effect to failure of the venopressor mechanism. Aub and Cunningham concluded that there was a markedly diminished oxygen content of the venous blood in experimental traumatic shock produced by crushing the hind legs of cats. This diminution occurred before the blood pressure fell to a shock level, and was still present after apparent recovery from shock. With this evidence of a local cause of shock to direct his efforts, Blalock (1930) hammered one hind leg in a number of dogs, thus reducing the blood pressure to a shock level, and noted that the injured side weighed from 530 to 960 Gm. more than the normal side. The increased weight of the injured limb was about 4 per cent of the body weight and was due to the extravasation of blood incident to trauma.

The importance of the loss of this amount of blood was proved by bleeding normal dogs 1 per cent of their body weight at intervals of an hour. In three or four hours, the systolic blood pressure of these dogs dropped to 70 mm. of mercury. In trauma to the intestine, the

average loss of body weight was 4.48 per cent—this mostly in the form of fluid devoid of red blood cells. In burns of one hind leg, the average increase in weight in the form of edema amounted to 3.38 per cent of the body weight. The loss of whole blood is apparently withstood better by a dog than the loss of either plasma or red cells alone. In types of shock studied by Blalock, it seemed that fluid from the blood is not reabsorbed to any marked degree because the osmotic pressure in the injured tissue is practically equivalent to that of the blood. The diminution in the capillary pressure probably tends to draw fluid into the blood stream in the noninjured regions. However, if this results, the increase in the mechanical pressure which it causes would tend to cause further loss of fluid into the injured tissues. "It is believed that the results of these experiments indicate that the loss of plasma protein is the most important factor in the production of the low blood pressure after the procedures reported." Previous work on plasma proteins (Whipple, Smith and Belt) established their great value and the fact that, once they have been depleted, their regeneration is a slow process requiring several days. Studies of plasmapheresis have proved that injured hepatic cells will predispose an animal to shock. Thus strong support is given the theory that there is a primary cellular injury that precedes the familiar clinical reaction of shock. Injuries of the pancreas, kidney and intestine do not, however, result in the development of such a predisposition when standard plasmapheresis is employed. A summary of the large number of experiments on shock by Blalock, Beard and Johnson appeared recently, and again emphasis was placed on the loss of whole blood or plasma as the major constant feature associated with shock. Parsons and Phemister found that the general symptoms developing in animals with traumatized limbs were practically the same as those that occur after hemorrhage. As an interesting incidental observation, incoagulability of the extravasated blood was reported. The reason for this behavior is not known, although search in extravasated blood and traumatized muscle for heparin failed to reveal this substance. The blood did not clot even when calcium salts or blood serum was added, although circulating blood removed from the heart before or after death clotted in normal time. Examination of the circulating blood of traumatized dogs disclosed increasing evidence of anemia which almost paralleled that in other dogs in which blood pressure had been reduced to a corresponding level.

Evidences of shock from loss of water after burns (Blalock, 1931; Underhill, Carrington and Kapsinaw; Underhill), influenza (Underhill and Kapsinaw), and a host of apparently unrelated perverted states of body, such as high fever, prolonged vomiting, as in intestinal obstruction, and severe diarrhea (Marriott), have served to stimulate investigation of the water content of blood and tissue in shock. Harris and

Blalock estimated the amount of water and solids of the liver, lung, kidney, intestine, heart muscle, brain, spleen and skeletal muscle before and after trauma to an extremity or to the intestine and after burns. There was little difference in the water or solid content after these procedures. In studies of the blood itself, a concentration of 21.5 per cent was noted after removal of plasma and after trauma to an extremity. The conclusions drawn from experiments in which depletion of plasma was produced are similar to those already arrived at by clinical observations. The plasma proteins withdrawn in the plasma or lost in a traumatized leg can no longer maintain the osmotic pressure in the capillaries or serve to counterbalance the force of filtration due to hydrostatic pressure. Loss of these colloids lowers the total osmotic pressure of the blood, and hence only a small amount of water passes into the blood stream. The same mechanism apparently is at work after hemorrhage and after shock. Associated with trauma is injury to the capillaries, which allows not only water but colloids to pass through their walls. The loss of large amounts of plasma causes a diminution in the capillary pressure, and this probably tends to allow fluid to pass into the blood stream in noninjured parts of the body as in hemorrhage. If this takes place, the increase in pressure in the capillaries probably causes further loss of fluid into the injured part. It is probably for this reason that shock due to trauma is usually more difficult to treat than that due to uncomplicated hemorrhage. After hemorrhage it is usually simply a matter of restoring the volume of the blood after the bleeding vessels have been ligated. After trauma, any procedure that increases the hydrostatic pressure in the vessels tends to cause further loss of fluid through the injured capillaries.

The latest reported investigations on the water balance in shock are those of Robinson and Parsons. They pointed out that blood is a liquid of which from 78 to 81 per cent is water, and that this water, according to recent views, is "bound" or modified by the colloids present in it. Their results are summarized as follows:

> . . . in hemorrhage, injection of histamine, and shock due to hemorrhage, an alternation exists between the water content of the blood and that of the skeletal muscles. That is, under these conditions, when an increase occurs in the water of the blood, a decrease takes place in the water of the muscles, and vice versa. The changes are almost simultaneous, and in some cases seem to be proportionate. Generalized muscular activity produces slight but frequent fluctuations in the water content of both blood and muscles, sometimes increasing and at other times decreasing the percentage of water present. The alteration noted above was not observed under these conditions.
>
> A hemorrhage of from 200 to 300 cc. of blood in dogs weighing from 10 to 14 Kg. produces an increase in the percentage of the total and free water contents of the blood. That this indicates a dilution of the blood is shown by the decrease in the erythrocyte count. A fall in the total, free, and bound water in the muscles accompanies the rise in the water of the blood.

In all dogs, after the traumatized leg has become markedly swollen there occurs an increase in the water of the blood, a decrease in that of the muscles distant from the trauma, and a decrease in the number of circulating erythrocytes indicating hemorrhage in the limb. The condition at the end of the experimental trauma shows water relations similar to those that follow hemorrhage.

COMMENT

The astounding variability in the results of investigations of traumatic shock can be accounted for in much of the earlier work by the variety of animals, anesthetics and procedures employed. Frogs, guinea-pigs and cats were used in the studies of capillaries. Dogs, cats, rabbits and horses were used in other phases of the problem. These animals were given various anesthetics: Some were given ether and morphine, some ether alone, and some nitrous oxide, ethyl carbamate or sodium barbital. Elaborate devices to retain body heat while procedures designed to produce shock were in progress were provided for some animals, whereas in other experiments no such provisions were made, and no record of temperature appears. The possibility that the anesthetic might alter the results of investigations of shock has occurred to many observers. Unfortunately the studies intended to settle this question have led to widely divergent views. Cattell (1923 [*a*]) concluded:

In normal cats, the inhalation of ether results in a sudden drop in arterial pressure which is quite temporary. As anesthesia deepens, the pressure gradually recovers until, by the time the eye reflex has disappeared, it may have returned to its original level. In the shocked animal, there is no recovery of the blood pressure after the primary fall, and the pressure continues to fall to zero even before the eye reflex disappears. Ether causes a contraction of the peripheral blood vessels due to a direct stimulation of the vasomotor center and a reflex to the fall in pressure resulting from a depression of the heart.

This cardiac depression is apparently responsible for the diminished alkali reserve that Raymond found in dogs subjected to ether anesthesia. Even more incriminating evidence against ether is supplied by Quigley and Lindquist, who were able to compress the thoracic part of the aorta by ligatures for two and one-half hours without producing shock in dogs when no anesthetic was used. Similar compression for as little as fifteen minutes under ether anesthesia, however, resulted in such marked respiratory difficulty that the ligature had to be released. Voicing a contrary opinion, Cannon did not think the anesthetic was important because his results were the same with ethyl carbamate as with ether. Cattell (1923 [*b*]) found that morphine does not accelerate the production of shock in cats and seems to prevent the usual reduction in alkali reserve incident to the low blood pressure in shock. Henderson argued that such conserving action by morphine is due to its depressive action on respiration.

The increase in blood nitrogen so frequently recorded in shock and assumed in many instances to be due to absorption of destroyed tissue can apparently be brought about simply by the altered metabolism that results from reduced blood pressure. The danger associated with reducing the blood pressure, even when it is far above normal, is generally appreciated in clinical medicine. The experiences of Harris and Platt are illustrative of this danger. They studied twenty-two patients with high blood pressure for a week on a rigid diet, and in the succeeding week gave them either injections of a proprietary "colloidal" sulphur solution or erythrol tetranitrate by mouth. During the period of reduced blood pressure in these patients, their excretion of water and their alkali reserve diminished, blood urea and nonprotein nitrogen increased, uric acid, phosphates and chlorides were retained, and cardiac output decreased. Such results achieved by recognized therapeutic measures indicated the importance of an adequate blood pressure in preserving metabolic equilibrium.

Wiggers used a photokymographic record obtained by a differential water manometer to study the circulatory changes in shock. He concluded that "the decreased venous pressure and consequent reduction in minute output is the predominant factor in the pronounced fall of arterial pressure during the progressive stage of shock. The dynamics of the circulation indicate clearly, however, that a reduction in peripheral arterial resistance initiates the fall of arterial pressure and the diminished filling of the arterial trunks before the effective venous pressure and cardiac discharge are reduced." These conclusions based on results obtained by the most modern instruments of precision were contradicted within a year by Erlanger, Gesell and Gasser. These dissenters, interpreting their results in terms of activity of the vasomotor center, stated that in the early stages of exposure of the intestines the tone of the center is usually increased. By the time the arterial pressure has fallen to 50 mm. of mercury, the tone of the center is invariably subnormal. The fact that vasoconstriction is the rule while the pressure is high, and that dilation occurs after the pressure has been low for some time, seems to indicate that exposure of the intestines tends to cause a fall in pressure. This tendency is combated by the vasoconstrictor center as long as arterial pressure is high enough to maintain a sufficient supply of blood to the centers. An explanation of the discord in the work of Wiggers and Erlanger and his co-workers was brought about in some measure by Forward and Perme, who maintained that Erlanger, Gesell and Gasser tested largely the reaction of the peripheral arterioles in shock, whereas Wiggers determined changes in peripheral resistance due in part to alterations in the caliber of the capillaries. They held that they had direct proof that the early changes in the curves of arterial pressure in shock are associated with reduced peripheral resistance, but

that the arterioles at this time are constricted. They concluded that the point of vasodilation in shock is in the capillaries rather than in the arterioles. The fallacy of basing the presence of shock on readings of blood pressure alone, as pointed out by Porter and Cope, is again emphasized. The importance of blood volume diminution is again emphasized and offers an explanation for the development of shock not only in an animal that has bled, but also in one that has not.

SUMMARY

Most of the features of shock can be produced experimentally by bleeding an animal or allowing blood to escape into traumatized tissues. After hemorrhage and in shock increased blood nitrogen and decreased alkali reserve seem dependent on decreased volume flow of blood. Loss of plasma proteins, especially when associated with injury to the liver, is at present accorded prime importance in the development of secondary shock.

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Notes and News

University News, Promotions, Resignations, Appointments, Deaths, etc.—The death of Rudolf Kraus, director of the Instituto Bacteriologico de Chile, Santiago, at the age of 63 is announced. In 1897, Rudolf Kraus, then in Vienna where he did most of his scientific work, demonstrated that specific antibacterial serums produced precipitates in sterile filtrates of cultures of corresponding bacteria. He was the first secretary-general of the International Society for Microbiology.

In the department of pathology of the University of Chicago Paul R. Cannon has been promoted to professor and Oran I. Cutler has been appointed assistant professor.

William Hailock Park, director of research laboratories of the health department of New York, has been awarded the public welfare medal of the National Academy of Sciences for eminence in the application of science to public welfare.

Florence B. Seibert, member of the staff of the Sprague Institute for Medical Research and assistant professor of biochemistry in the department of pathology of the University of Chicago, has been appointed assistant professor of biochemistry in the Phipps Institute of the University of Pennsylvania.

William H. Howell has been elected chairman of the National Research Council for 1932-1933.

Stanhope Bayne-Jones has assumed the chairmanship of the division of medical sciences of the National Research Council for 1932-1933.

James L. Alloway has been appointed assistant professor of bacteriology in Cornell University Medical College.

In the University of Wisconsin Robert C. Bunts has been made assistant in pathology and Philip Levine instructor in bacteriology.

American Journal of Parasitology.—This journal, founded by Henry B. Ward, professor of zoology in the University of Illinois, under whose editorship eighteen volumes have been published, has been given by Professor Ward to the American Society of Parasitology. Henceforth the journal will be published as the official organ of the society, under the direction, for the next five years, of W. W. Cort, R. W. Hegner and F. M. Root, editorial committee.

International Cancer Research Foundation.—This foundation, which was organized on June 8, 1932, has been endowed with $2,000,000 by William H. Donner, Villanova, Pennsylvania. The object of the foundation is to "increase interest in and the amount and quality of cancer research; develop new minds and theories; broaden the viewpoint of some investigators already in the field, and increase cooperation among scientists throughout the world, correlating results of their investigations and preventing duplication of work." The scientific advisory committee consists of James Ewing, New York; Burton T. Simpson, Buffalo, and Francis Carter Wood, New York.

Abstracts from Current Literature

Experimental Pathology and Pathologic Physiology

RENAL THRESHOLD FOR HEMOGLOBIN IN DOGS UNINFLUENCED BY MERCURY POISONING. W. H. HAVILL, J. A. LICHTY, JR., G. B. TAYLOR and G. H. WHIPPLE, J. Exper. Med. **55**:617, 1932.

The minimal, or depression, renal threshold for hemoglobin in the dog is not modified by moderate doses of mercuric chloride. This type of renal injury involves the epithelium of the convoluted tubules, but the glomeruli escape. We are unable to explain our findings if we assume that the tubular epithelium takes an active part in the passage of hemoglobin from the blood into the urine. The evidence points toward the glomerular tuft as responsible for its passage from the blood plasma into the tubules. The glomerular tuft establishes the true hemoglobin threshold under these conditions. If the convoluted tubules are normal, we note that hemoglobin is taken into the epithelium, and this explains the high initial renal threshold. With repeated injections of hemoglobin, this tubular epithelium becomes stuffed with hemoglobin pigment fractions and can absorb no more, which explains the minimal or depression threshold. Further injury of this tubular epithelium with mercury causes no change in this minimal renal threshold, unless we produce actual tubular obstruction.

AUTHORS' SUMMARY.

TOLERANCE FOR MERCURY POISONING INCREASED BY FREQUENT HEMOGLOBIN INJECTIONS. W. H. HAVILL, J. A. LICHTY, JR., and G. H. WHIPPLE, J. Exper. Med. **55**:627, 1932.

Frequent injections of superthreshold amounts of canine hemoglobin in dogs will cause deposits of pigment in the renal tubular epithelium. When this has happened, the dog will survive minimal lethal doses of mercuric chloride with little evidence of renal injury. In fact, some dogs will tolerate twice the minimal lethal dose without severe reaction. There is no evidence that continued injections of hemoglobin in these amounts will cause any injury or functional disability of the kidney. Rest periods will effect a disappearance of this pigment in the renal tubules. [See also articles by W. V. Newman and G. H. Whipple and by R. P. Bogniard and G. H. Whipple, J. Exper. Med. **55**:637 and 653, 1932.]

AUTHORS' SUMMARY.

EFFECTS OF PARATHORMONE AND AMMONIUM CHLORIDE ON THE BONES OF RABBITS. H. L. JAFFE, A. BODANSKY and J. E. BLAIR, J. Exper. Med. **55**:695, 1932.

A very large single dose of parathormone produced rapid and extensive decalcification of the bones of a young rabbit. Gradually increased doses of parathormone failed to produce significant changes in the bones of young and fully grown adult rabbits. Ammonium chloride, administered by stomach tube, was without effect on the bones, when given to rabbits treated with parathormone or to otherwise untreated rabbits. Calcium lactate did not promote the appearance of signs of parathormone overdosage in rabbits during treatment, nor were metastatic calcifications observed in the soft tissues after termination of the experiment. The rabbit is relatively very resistant to the decalcifying effects of gradually increased, repeated doses of parathormone, and therefore is not satisfactory material for experimental studies of the bone changes of chronic hyperparathyroidism. The

appearance of actively transforming bones of a young, growing rabbit, particularly at the sites of rapid growth, is not to be confused with that of the more severe fibrous processes produced by parathormone in guinea-pigs, dogs and rats.

AUTHORS' SUMMARY.

EFFECT OF ANTITESTICULAR SERUM ON THE ENHANCEMENT VALUE OF TESTICLE EXTRACT. F. DURAN-REYNALS, J. Exper. Med. **55**:703, 1932.

The experiments reported here show that the infection-enhancing factor of testicle extract is neutralized in vitro by an antiserum against homologous testicle extract. An antiserum developed against a testicle extract of one species does not influence the enhancing and spreading factor of the extract from another species. Rabbits immunized against testicle extract do not exhibit any alteration in the spreading or enhancing effect of extracts employed later, even when the testicle extract used is from the same species as that employed for the immunization. Whether the in vitro inactivation of the active factor is a specific neutralization, or is the result of adsorption of the factor on the flocculate formed, has not been definitely determined. The fact that there is no neutralization in vivo, and that the antiserum acts only on extracts from the same species, when definite flocculation takes place, tends to emphasize the probability that the neutralization is not a direct one, but is incidental to the flocculation mentioned.

AUTHOR'S SUMMARY.

THE RACES OF COMMON FIBROBLASTS. R. C. PARKER, J. Exper. Med. **55**:713, 1932.

The ability of fibroblasts to mature and to manifest their various potencies in any particular medium is inversely proportional to the growth energy which they exhibit in that medium. Fibroblasts having access to high concentrations of food substances in their environment do not mature, regardless of their origin or of the age of the animal from which they were derived. They behave as embryonic cells. Fibroblasts cultivated in vitro are potentially able to produce cells with the structural and functional properties commonly attributed to macrophages. This is true regardless of their origin or of the length of time that has elapsed since their isolation from the origin. The fibroblasts and the macrophage are considered to represent extreme functional and structural variations of the same cell type. The structural and functional characteristics displayed by fibroblasts in vitro vary according to their origin and according to the changes that take place in the composition of the medium in function of time.

AUTHOR'S SUMMARY.

MOBILIZATION AND EXCRETION OF CALCIUM FOLLOWING OVERDOSAGE WITH IRRADIATED ERGOSTEROL. N. B. TAYLOR and C. B. WELD, Brit. J. Exper. Path. **13**:109, 1932.

The hypercalcemia following large doses of viosterol is due to withdrawal of calcium from the skeleton. Viosterol overdosage depresses the power of the intestine to excrete calcium. The calcium mobilized from the bones accumulates in the serum. A certain level of serum calcium having been reached, an increase in the urinary calcium follows. Viosterol in the amounts that produce hypercalcemia does not increase absorption of calcium from the intestine.

AUTHORS' SUMMARY.

EFFECT OF VITAMIN DEFICIENCY ON THE STRUCTURE OF THE THYROID AND THYMUS GLANDS. A. W. SPENCE, Brit. J. Exper. Path. **13**:157, 1932.

An attempt was made to produce lymphadenoid goiter in twenty-four young rats by means of a diet consisting of: white flour, 82 per cent; olive oil, 8 per cent;

meat residue, 5 per cent; salt mixture (including an adequacy of potassium iodide), 5 per cent, and an unlimited supply of distilled water. The animals were on the diet for from nine weeks to thirteen months. Simple goiter was produced in fifteen of twenty rats examined, of which eight had slight goiters, five small goiters and two large ones. Lymphadenoid goiter did not arise. The production of simple goiter was independent of an absolute iodine deficiency. The factors concerned were dietetic insufficiency and infection. Outstanding was the occurrence of keratinization cysts in the thyroid gland in twenty-three of the twenty-four animals. These cysts arose from the vesicles, probably as a result of exhaustion. Similar cysts were found in the thymus glands of three animals on the diet, and are considered to be large Hassall's corpuscles and not an effect of vitamin deficiency. No support was found for the hypothesis that the cysts in the thyroid and thymus glands represent distentions of the thyroid-thymic lymphatics.

AUTHOR'S SUMMARY.

THE TOXICITY OF IRRADIATED ERGOSTEROL. J. B. DUGUID, M. M. DUGGAN and J. GOUGH, J. Path. & Bact. **35**:209, 1932.

Viosterol was found to be more toxic in rats fed on a certain synthetic vitamin-free diet of high calcium content than in rats on a normal diet. The absence of vitamins and the high calcium content were considered as possible determinants of the toxicity, and the high calcium content was found to be the more powerful. After reduction of the calcium and also of the phosphorus content of the diet, the evidence suggested that the absence of vitamins also contributed, but the evidence of this is not accepted as convincing. The toxicity was estimated chiefly on arterial and renal calcification. The reliability of these lesions as criteria is discussed. Arterial lesions are accepted as reliable indications of viosterol poisoning in the rat. Renal lesions were noted to occur in viosterol poisoning in two forms, namely, calcification of the renal arterioles, and calcareous casts in the renal tubules. The former appeared as part of a general arterial involvement and, as such, was taken as a reliable indication. The latter was found to be a lesion of too common occurrence in the rat, independently of the administration of viosterol, to be admitted as reliable evidence of toxicity.

AUTHORS' SUMMARY.

FOETAL RICKETS. J. P. MAXWELL, C. H. HU and H. M. TURNBULL, J. Path. & Bact. **35**:419, 1932.

The present case establishes beyond doubt the occurrence of rickets or of osteomalacia in the fetus. The relation of the two diseases is discussed in the light of recent investigations. It is concluded that both are metabolic, are doubtless due usually to food deficiencies and are probably both due to a deficiency of vitamin D. Their causal identity is not, however, proved. Although, therefore, the two cases bring additional evidence of the histologic identity of osteomalacia and rickets, they do not prove the identity of the two diseases.

AUTHORS' SUMMARY.

Pathologic Anatomy

CONGENITAL MEDIAL SCLEROSIS OF THE CORONARY ARTERY. R. W. KISSANE and R. S. FIDLER, Am. Heart J. **7**:133, 1931.

The literature is reviewed, and a case is described. It concerns a noninflammatory primary thickening of the media of the larger coronary branches. The electrocardiogram shows a T-wave like that seen in the more familiar coronary sclerosis.

GONOCOCCUS ENDOCARDITIS. HENRY B. KIRKLAND, Am. Heart. J. **7**:360, 1932.

A case of gonococcic endocarditis with autopsy is reported. Acute endocarditis followed urethritis and affected an aortic valve already damaged by rheumatism. In addition to acute vegetative endocarditis of the aortic valve, autopsy revealed chronic productive changes in both mitral and aortic valves, a subendocardial abscess with extension to the pericardium, diffuse suppurative and hemorrhagic pericarditis, acute intracapillary glomerular nephritis, acute focal interstitial nephritis, parenchymatous degeneration of the kidneys, infarction and suppuration of the spleen, degeneration and congestion of the liver, suppurative gastritis, pulmonary congestion, bilateral hydrothorax, ascites, conjunctival petechiae, suppurative leptomeningitis, gangrene of the legs and a decubitus ulcer.

AUTHOR'S SUMMARY.

AN UNUSUAL CASE OF LEFT-SIDED DISPLACEMENT OF THE HEART. LOUIS H. SIGLER, Am. Heart J. **7**:388, 1932.

A case of sinistrocardia, most likely congenital, is reported, which from perusal of the literature appears to be the only one of its kind on record. The outstanding features in the case were: 1. A healthy man, 50 years old, had gone through life without any circulatory or respiratory embarrassment. 2. The heart with its basal vessels was entirely displaced to the left of the spinal column. 3. Other mediastinal contents, such as the esophagus, trachea and bronchi, were likewise displaced. Although a history of injury was given, the injury seems to have been a mere coincidence, as there was no definite evidence of the presence of a pathologic condition that might be considered to be the cause of the displacement.

AUTHOR'S SUMMARY.

MONONUCLEAR ERYTHROPHAGOCYTOSIS IN THE BLOOD OF A NEW-BORN INFANT. A. F. ABT, Am. J. Dis. Child. **42**:1364, 1931.

Abt briefly reviews the conditions in which mononuclear cells have been noted to phagocytose red blood cells. These conditions have invariably been pathologic and include paroxysmal hemoglobinuria, pernicious and secondary anemia, malarial infestation, sepsis and tuberculosis. This unusual phagocytic activity also occurs after splenectomy and most commonly is seen in endocarditis lenta. Abt observed this activity in an infant, aged 9 days, suffering from anemia of the new-born. Such erythrocyte-laden cells were observed for five days and then they disappeared. The phagocytic cells in this child were small macrophages, which seemed to have developed through the progressive hypertrophy of lymphocytes and monocytes.

GEORGE RUKSTINAT.

MIGRATORY PERITONITIS (SO-CALLED HEMATOGENOUS PERITONITIS) IN CHILDREN. S. A. WILE and O. SAPHIR, Am. J. Dis. Child. **43**:610, 1932.

Twenty-four cases of peritonitis, twenty-one of which were diagnosed clinically as hematogenous peritonitis, proved at autopsy to be the result of an invasion of the peritoneal cavity by bacteria from primarily diseased intraperitoneal organs or structures close to the peritoneum, without perforation or without direct extension of the inflammatory process to the peritoneum. Sixteen of the twenty-four cases showed enteritis. The term "migratory peritonitis" seems appropriate for this type of case. Clinical reports of cases of hematogenous peritonitis without autopsies, or with autopsies but without complete histologic examination of the gastro-intestinal tract, such as comprise the bulk of the literature of so-called hematogenous peritonitis, should be discarded. Many of the cases in which the condition was reported as hematogenous peritonitis are examples of migratory peritonitis. The evidence supporting the occurrence of peritonitis directly following infection of the blood stream without an intermediary lesion

within or adjacent to the peritoneal cavity is inadequate. From our observations it is apparent that so-called hematogenous peritonitis may occur as part of a generalized metastatic pyemic process that results in abscess formation or infected thrombi within or adjacent to the peritoneal cavity. The ensuing peritonitis is the direct result of the abscess or thrombus. It is unlikely, however, that this form of peritonitis occurs in the absence of other metastatic pyemic phenomena.

AUTHORS' SUMMARY.

THE NORMAL HISTOLOGY OF INFANTS' BRAINS. HERMAN SCHWARZ, P. GOOLKER and JOSEPH H. GLOBUS, Am. J. Dis. Child. **43**:889, 1932.

Some well established, but not widely known, anatomic features characterizing certain stages in the developing brain are described. They often assume the form of circumscribed collections of deeply staining cells, which are often erroneously considered of inflammatory origin. Nine cases of intestinal intoxication are described. They revealed no evidence of an inflammatory or degenerative disease in the brain. Evidence is presented against a recent view that an encephalitic process in the diencephalon, affecting certain hypothalamic centers, causes the dehydration of intestinal intoxication. The conclusion is reached that no pathologic alterations were found in the brains studied to account for the clinical picture in intestinal intoxication.

AUTHORS' SUMMARY.

RHEUMATIC PNEUMONIA. B. A. GOULEY and J. EIMAN, Am. J. M. Sc. **183**:359, 1932.

Eight of nine cases of acute rheumatic fever showed an acute inflammation of the pulmonary tissue, with consolidation; the ninth showed pleurisy, with subacute pulmonary involvement. All were associated with acute rheumatic disease of the heart. The inflammatory pulmonary reaction consisted of an interstitial perivascular exudate of large endothelioid cells, morphologically identical with those found in rheumatic lesions of the heart and considered pathognomonic of rheumatic fever. Hemorrhage and fibrinous exudate were prominent features. We therefore consider that in many virulent cases a characteristic rheumatic pneumonia is to be found.

AUTHORS' SUMMARY.

DIABETIC GANGRENE. DAVID W. KRAMER, Am. J. M. Sc. **183**:503, 1932.

Various types of gangrene may occur in diabetes. The arteriosclerotic type and the so-called diabetic gangrene are the most common. Thrombo-angiitis obliterans and embolic gangrene are less so. The advisability of recognizing diabetic gangrene as a definite form is discussed. The fact that a certain number of diabetic persons with gangrene present clinical features and pathologic changes in the vessels, plus the fact that the gangrene may be of the moist variety, lends support in deciding that one is dealing with a form other than the well accepted arteriosclerotic type of gangrene. Analysis of 1,008 cases of diabetes mellitus observed between 1920 and 1930 shows that there were 58 cases of existing gangrene (5.75 per cent), 28 cases of threatened or impending gangrene and 89 cases of potential gangrene. Collectively, the three groups totaled 175 cases (17.3 per cent) of circulatory disturbances. If the more recent five year period (1926 to 1930) is considered, the incidence is higher: 6.78 per cent showed gangrene, and 19.75 per cent, deficient circulation in the extremities. My figures compare closely with the average statistics of others. Among 12,037 patients with diabetes, there were 756 cases of gangrene (a percentage of 6.28). Diabetic gangrene is the end-product of three main influences: the metabolic disturbances and their effect on the vessels and the tissues, changes in the arteries, with the resulting deficient blood supply to the parts, and infection.

AUTHOR'S SUMMARY.

THE PATHOGENESIS OF PERIGASTRIC ABSCESS COMPLICATING PEPTIC ULCER. HARRY A. SINGER and PETER A. ROSI, Am. J. M. Sc. **183**:600, 1932.

In twenty-nine of thirty cases of perigastric (including subphrenic) abscess, with origin in ulcer, convincing evidence was obtained of a previous acute perforation into the free abdominal cavity. The formation of the adhesions that are found about a perigastric abscess generally follows rather than precedes perforation. Between the time of recovery from the acute symptoms of perforation and the time of onset of manifestations of a perigastric abscess a period of fair health frequently intervenes. It is on this account that a patient often fails to associate the two illnesses. Unless a previous acute perforation is suspected and a minute-by-minute history of the initial symptoms obtained, information of the antecedent occurrence of a sudden, violent abdominal pain is often not elicited. In most instances, a perigastric abscess of ulcerative origin represents a neglected opportunity on the part of the patient, but perhaps more frequently on the part of the physician. Early recognition of perforated peptic ulcer, especially the type with mild postperforative symptoms, together with timely operation, will eliminate the greater number of perigastric abscesses.

AUTHORS' SUMMARY.

CHANGES IN POLYMORPHONUCLEAR LEUKOCYTES IN INFECTIONS. M. A. KUGEL and NATHAN ROSENTHAL, Am. J. M. Sc. **183**:657, 1932.

A study of the blood in various infections shows marked variation in the number of leukocytes and in the number of nonsegmented and segmented polymorphonuclear cells. In certain infections, the polymorphonuclear leukocytes show cytoplasmic granular changes. The changes include marked basophilia and, less frequently, vacuolization of the cytoplasm and appearance of certain cytoplasmic masses known as Döhle's bodies. The basophilic, or "toxic," granules are seen only in infections — most often in bacteremias. In streptococcic infections they occur late, particularly in subacute endocarditis; but in other conditions, especially those associated with more or less extensive suppuration (as pneumonia), they make their appearance early and usually persist for weeks after the crisis. "Toxic" granules occur in both segmented and nonsegmented polymorphonuclear neutrophils. The absence of "toxic" granules is suggestive of mild or localized infection. Persistence of this condition indicates a good prognosis or absence of complications. Absence of "toxic" granules or of degenerative changes in the cytoplasm of the polymorphonuclear leukocytes in cases of severe infection is unusual. The degenerative index offers a prognostic guide in infectious conditions: A decrease indicates recovery; a steady increase suggests either a complication or a bad prognosis; an index over 90 per cent is usually associated with severe infection, regardless of the clinical condition, and is unfavorable. Frequent observations of nuclear changes, such as segmentation and nonsegmentation, combined with consideration of cytoplasmic changes, is a most valuable aid in gaging the severity and the course of an infection.

FROM AUTHORS' SUMMARY.

ANATOMICAL CHANGES IN THE LIVERS OF DOGS FOLLOWING CONSTRICTION OF THE HEPATIC VEINS. J. P. SIMONDS and J. W. CALLAWAY, Am. J. Path. **8**:159, 1932.

The livers of dogs examined twenty-four, forty-eight and seventy-two hours and seven days after mechanical obstruction of the hepatic veins for periods of from seven to thirty minutes showed the following changes: a mean increase of 25 per cent in the liver weight-body weight ratio, due to edema and to swelling of the hepatic cells; swelling, granulation, vacuolization and extensive necrosis of the hepatic cells in the central half or two thirds of the hepatic lobules; marked dilatation of the perivascular lymphatic vessels surrounding the sublobular veins; the presence of hyaline thrombi in many central and sublobular veins;

intrasinusoidal cell masses of two types, i. e., small, compact, occluding masses probably originating in "conglutination thrombi" of red cells, and larger, more diffuse, branching cell masses; hemosiderosis of Kupffer cells.

AUTHORS' SUMMARY.

EXPERIMENTAL MUSCLE DEGENERATION. D. K. and H. R. FISHBACK, Am. J. Path. **8**:193 and 211, 1932.

Degeneration of skeletal muscle has been produced by physical, chemical, bacterial, parasitic and pharmacologic trauma. The stages of degeneration produced were: slight granular clouding and swelling, with dimming of cross-striations; edema of fibers with prominent longitudinal fibrils; vacuolization; true granular degeneration, either albuminous or fatty; waxy degeneration with, further, lumpy disruption or granular disruption. The name "acute molecular degeneration of striated muscle" is suggested as a better descriptive and more inclusive term than "waxy degeneration."

Acute molecular degeneration of striated muscle in rabbits is a progressive process, including edema, fibrillar separation, vacuolization, hyaline change, lumpy disruption, granular change and finally complete dissolution of the muscle cytoplasm. This is associated with some cell exudation, with a high degree of phagocytic activity, and finally with repair. The course of repair is toward regeneration. The completeness of this process appears to depend on the destructiveness of the lesion, and not on the extent or the severity of the degeneration of muscle fibers. If the destruction of the sarcolemma is not too severe, and if the stroma remains, these, with surviving muscle nuclei, form the integral factors for muscle restoration With diffuse destruction of tissue, scarring results.

AUTHORS' SUMMARIES.

VITAL STAINING OF THE RABBIT'S AORTA IN THE STUDY OF ARTERIOSCLEROSIS. G. L. DUFF, Am. J. Path. **8**:219, 1932.

In rabbits, intravenous injection of a suitable quantity of a solution of trypan blue results in well marked staining of the wall of the aorta within sixteen hours. The depth of color as seen on the intimal surface is not uniform, some areas being more deeply stained than others. The differences in intensity of staining become more prominent with increase in the length of the experiment. The variations are the result of irregularities in the staining of the outer layers — the adventitia and the outer portion of the media. The deeply staining areas correspond to the areas in which the aortic wall is most plentifully supplied by vasa vasorum, while the pale staining areas correspond to those in which the vascularization of the aorta is least abundant. The staining of the wall of the aorta is chiefly due to the escape of the dye through the capillary endothelium, which is much more permeable to trypan blue than is the lining endothelium of the aorta. The local variations in depth of staining in the aorta are thus dependent on the degree of vascularization of its walls. The production of an inflammatory reaction in the external layers of the aorta brings about a local increase in capillary permeability to trypan blue and as a result a stronger staining of the wall in the inflamed area.

AUTHOR'S SUMMARY.

THE QUESTION OF A SPECIFIC MYOCARDIAL LESION IN HYPERTHYROIDISM. W. LEWIS, Am. J. Path. **8**:255, 1932.

In some cases of hyperthyroidism, certain degenerative and inflammatory changes that occur in the myocardium indicate a toxic origin and suggest a toxin circulating in the blood stream. Two cases showing such myocardial changes are reported.

AUTHOR'S SUMMARY.

ATELECTASIS AS A FACTOR IN THE EVOLUTION OF CHRONIC FIBROID PULMONARY TUBERCULOSIS. HERMAN HENNELL, Am. Rev. Tuberc. **23**:461, 1931.

In so-called "advanced unilateral fibroid phthisis," massive atelectasis of large portions of pulmonary tissue, occasionally involving most of one lung, is probably the essential mechanism. The tuberculous process in the lung may be minimal, and its clinical significance may become relatively unimportant. The marked pulmonary fibrosis, which rapidly develops after atelectasis occurs, may submerge the tuberculous process, and the bronchiectasis that follows in the wake of the pulmonary fibrosis may determine the clinical features and course. In the evolution of fibroid phthisis of lesser degree, atelectasis involving smaller or larger areas of pulmonary tissues is probably an important factor. It is conservative, and may be responsible for the mild clinical course as well as the slow progress of the pathologic process after it is established.

H. J. CORPER.

ATELECTASIS IN PULMONARY TUBERCULOSIS. E. KOROL, Am. Rev. Tuberc. **23**:493, 1931.

Atelectasis is common in tuberculosis and is responsible for contraction of the chest and lung, limited mobility, feeble or absent vesicular murmur and decreased complemental space. The small lung with a displaced mediastinum and diaphragm frequently develops in spite of the absence of fibrosis and adhesions; it is due to a diminished amount of air in the lung and a fall in intrapleural pressure. The pendulum movement of the mediastinum observed in bronchostenosis and atelectasis occurs also in tuberculosis with preponderantly unilateral distribution. Paradoxical breathing accompanies tuberculous disease of the lung; it is believed to be the cause of the characteristic râles. In many cases, this breathing from one lung into the other spreads the tuberculous infection.

H. J. CORPER.

PULMONARY ASBESTOSIS. K. M. LYNCH and W. A. SMITH, Am. Rev. Tuberc. **23**:643, 1931.

Lynch and Smith have collected reports of 172 cases of pulmonary asbestosis. In 27, the diagnosis was confirmed by the finding of asbestosis bodies in the sputum or in the lung juice obtained through puncture or by necropsy. Necropsy was made in 18 cases. In 3 of these, the disease was complicated by pulmonary tuberculosis; in 3, by lobar pneumonia, and in 3, by bronchopneumonia. One case was terminated by a traumatic death. In 4, a complete report was lacking, but the statement was made that necropsy confirmed the diagnosis. Including the very first report of a case, that of Murray in the *Charing Cross Gazette* of 1900, which apparently received little attention until it was resurrected by Cooke, there are now 4 records of necropsy on uncomplicated pulmonary asbestosis.

H. J. CORPER.

CHOLESTEROL-THORAX IN TUBERCULOSIS (CHOLESTEROL PLEURISY). HARRY M. STEIN, Arch. Int. Med. **49**:421, 1932.

The case reported is one of cholesterol pleurisy occurring in a man who had a history of pleural effusion following pneumonia. In the absence of a clearer history it might be that the condition was originally one of tuberculous pleurisy with effusion and not of pneumonia. The patient had tuberculosis involving the central nervous system many years after his probable tuberculous pleurisy, and there was postmortem evidence of tuberculosis of the pleurae. Cholesterol-thorax might be looked on, in the majority of instances, as an unusual end-result of a long-standing pleural effusion that was originally tuberculous. One might assume that, in view of the fact that the majority of primary pleural effusions are tuberculous in origin, cholesterol-thorax simply means that an old tuberculous effusion

which has not been cured and which has not caused death will, in some instances, eventually become cholesterolized. Cholesterol metabolism is, of course, as yet an unsolved problem, and it may be that an old pleurisy becomes cholesterolized only in a person who has an abnormal cholesterol chemistry, this observation, of course, being theoretical. Reports vary as to the cholesterol chemistry in pulmonary tuberculosis; it would seem that the cholesterol content in the blood in tuberculous infections may be increased or decreased. Eichelberger and McCluskey claimed that a rising or stationary hypercholesteremia indicates immunity and resistance, while a falling or low cholesterol content of the blood indicates a lowering in immunity and resistance.

AUTHOR'S SUMMARY.

THE STUDY OF CONCRETIONS BY X-RAYS. K. H. BAUER, Verhandl. d. deutsch. path. Gesellsch. **26**:299, 1931.

Roentgen examination of concretions, inside and outside of the body, is simple and reliable and gives almost as much information as study of microscopic sections. Since the different substances composing the stones have entirely different densities, a diagnosis of the chemical structure can be obtained in most instances from x-ray pictures, before removal of the calculi from the body. In 11.6 per cent of 250 patients with multiple gallstones, fragmentation of concretions, leading to new formation of stones, could be demonstrated with this method. The intact stones showed clefts as the first stage of fragmentation. The physical process of fragmentation is explained on the basis of the view of Goldschmidt.

C. ALEXANDER HELLWIG.

CHRONIC PASSIVE CONGESTION OF THE SPLEEN. E. JAEGER, Verhandl. d. deutsch. path. Gesellsch. **26**:334, 1931.

Chronic passive congestion of the spleen was produced in dogs, and the morphologic changes in the spleen were studied. Continuous congestion caused induration characterized by marked thickening of the trabeculae and of the capsule, dilatation of the trabecular veins and atrophy of the malpighian bodies. Remittent congestion produced hyperplasia and dilatation of the sinus, thickening of the reticulum and moderate fibrosis of the pulp and malpighian corpuscles. Furthermore, the author observed siderofibrosis, so-called Gandy-Gamna's nodules, in the wall of larger blood vessels of the trabeculae and in the capsule. Jaeger concludes that splenic tumor accompanying cirrhosis of the liver may be due to passive congestion alone, and he rejects the opinion of many writers that splenomegaly in cirrhosis of the liver is due to a toxic agent influencing simultaneously the spleen and the liver.

C. ALEXANDER HELLWIG.

PANCREATIC FERMENTS IN THE BILE OF THE GALLBLADDER. A. WERTHEMANN, Verhandl. d. deutsch. path. Gesellsch. **26**:343, 1931.

The average content of amylase in the bile of fifty-one gallbladders was 312 (Wohlgemuth's method). Amylase is apparently not secreted by the mucosa of the gallbladder, since it is practically absent in cases of obstruction of the cystic duct. When there is a communication between the pancreatic and the common bile duct, pancreatic juice may enter into the bile ducts. This, however, does not by itself cause bilious peritonitis without perforation, because in such cases, it was found, the amylase content of the bile is much lower than that in the pancreatic juice. Also the bilious imbibition of the surrounding tissue of the gallbladder, as found at autopsies, is without relation to the amylase content of the bile, but depends on chronic inflammatory fibrosis of the wall of the gallbladder and on the viscosity of the bile.

C. ALEXANDER HELLWIG.

LARGE GROUND PREPARATIONS OF THE VERTEBRAL COLUMN. L. MICHAELIS, Verhandl. d. deutsch. path. Gesellsch. **26**:363, 1931.

The principle of Christeller's large sections of parenchymal organs is applied to the study of lesions of the bones. Portions of the vertebral column, consisting of from four to eight vertebrae, the half of the femur and other long bones are sawed longitudinally into halves and one of them is ground and polished. The thin preparations can be studied microscopically by reflected light. Photomicrographs are demonstrated which were made from these sections, with a magnification of 1,000 diameters. This new method permits the histotopographic study of lesions of the bones and is of great practical value.

C. ALEXANDER HELLWIG.

THE ARCHITECTURE OF THE ASCENDING AORTA AND ITS PATHOLOGIC SIGNIFICANCE. F. ORSÓS, Verhandl. d. deutsch. path. Gesellsch. **26**:365, 1931.

The study is based on 15,000 serial sections from 200 aortae. A system of muscular-elastic bundles is described that shows a typical arrangement in the wall of the ascending aorta. The longitudinal bundles of this system and the margins of the sinus are predisposed to atherosclerosis. It is in these structures that histologic examination often reveals definite atherosclerotic changes, while macroscopically the aorta still appears completely normal.

C. ALEXANDER HELLWIG.

PATHOLOGIC ANATOMY OF THE SALIVARY GLANDS. E. KIRCH, Verhandl. d. deutsch. path. Gesellsch. **26**:387, 1931.

For the first time, invasion of the excretory ducts of salivary glands by pyogenic cocci has been demonstrated in cases of septicemia and pyemia. Often small hyaline or white thrombi were found in blood vessels of the glands, but never abscesses in the glandular parenchyma. Arteriosclerosis is a frequent finding in salivary glands and accompanies most cases of arterial hypertension. Atherosclerosis of the larger arteries, however, is uncommon. Glycogen was found in all salivary glands in cases of sudden death and seems to be normal in these glands. Brown pigmentation of the epithelial cells of the intercalated ducts is common in older people.

C. ALEXANDER HELLWIG.

PHYSIOLOGIC TRANSFORMATION OF GASTRIC GLANDS. H. HAMPERL, Verhandl. d. deutsch. path. Gesellsch. **26**:392, 1931.

In 400 organs fixed immediately after death, the structure of the fundic glands was studied with special staining methods (methylviolet and Best's carmine stain). The zymogenic cells can slowly disappear during the physiologic process of replacement of shed cells and can be replaced by mucous neck cells, until finally the whole fundic gland consists of goblet cells. The glands found after this physiologic process cannot be distinguished from the pseudopyloric glands due to regeneration of defects of the mucosa.

C. ALEXANDER HELLWIG.

Microbiology and Parasitology

THE EARLY CELLULAR REACTIONS IN THE LUNGS OF RABBITS INJECTED INTRAVENOUSLY WITH HUMAN TUBERCLE BACILLI. ARTHUR J. VORWALD, Am. Rev. Tuberc. **25**:74, 1932.

The cells that respond first to an intravenous injection of tubercle bacilli of the human type into rabbits (0.1 mg. per kilogram of animal weight) are the polymorphonuclear leukocytes. This reaction, while transitory, is not one of negligible significance. It is through the agency of the polymorphonuclear leukocytes that the site of subsequent development of the tubercle is determined. These

cells phagocytose the tubercle bacilli in the blood stream and stagnate in small accumulations in the capillaries where the current is slow. In this way, injected bacilli are concentrated and localized. The focal accumulations of polymorphonuclear leukocytes form the groundwork from which the epithelioid tubercles develop. Mononuclear exudate cells are preponderant in the cellular response after the first few hours (from fourteen to eighteen hours on). They mingle with the polymorphonuclear leukocytes, phagocytose and digest them and take up their content of tubercle bacilli. In this way, by replacement of the leukocytic mass, the mononuclear cell tubercle, ultimately the epitheloid tubercle, develops. The increase in mononuclear exudate cells in the field of inflammation, in almost total absence of mitotic figures in such foci, indicates that the majority of reacting cells of this type are hematogenous in origin.

APPENDICEAL OXYURIASIS. H. GORDON, Ann. Int. Med. **4**:1521, 1931.

Gordon investigated the occurrence of oxyurids in 20,969 appendixes that had been received for routine diagnosis at the pathologic laboratories of the University of Michigan from 1894 to 1930. Only surgical specimens were considered. Oxyurids were found in 221 appendixes (1.05 per cent). Infestation was found to be much more common during the first decade of life and almost entirely absent after the forty-sixth year. There was no significant seasonal predilection for the occurrence of oxyuriasis. The significantly greater incidence of appendical oxyuriasis during the past decade is probably related to the increased consumption of uncooked leafy vegetables and raw fruits. WALTER M. SIMPSON.

EXPERIMENTAL AGRANULOCYTOSIS (SALMONELLA SUIPESTIFER). B. M. FRIED and WILLIAM DAMESHEK, Arch. Int. Med. **49**:94, 1932.

The purpose of the present study has been to determine the possible similarity between the blood picture as seen in agranulocytosis in man and that found in a form of experimental sepsis in rabbits. The results have shown that there are close similarities between the agranulocytosis resulting from the hematogenous infection of rabbits with Salmonella suipestifer and that observed in cases of agranulocytic angina in man. Thus, the reaction in severe cases of human agranulocytosis corresponds to that of the animals that received overwhelming doses of bacteria, i. e., persistent neutropenia and intense necrosis of the bone marrow without signs of regeneration. A close similarity likewise exists between the "recovery phase" seen in the circulating blood in clinical agranulocytosis and that disclosed in the circulation of rabbits that were infected with relatively small doses of bacteria, i. e., marked histiomonocytosis. Incidentally, Schilling's clinical concept of "regenerative" and "degenerative" types of polymorphonuclear "shift" was confirmed by these experiments. AUTHORS' SUMMARY.

THE EFFECT OF SODIUM RICINOLEATE ON THE GONOCOCCUS. C. PHILLIP MILLER, JR., and R. CASTLES, J. Bact. **22**:339, 1931.

Sodium ricinoleate does not "detoxify" gonococci but rather increases their lethal action for mice. It does not destroy the antigenic properties of gonococci. AUTHORS' SUMMARY.

COLONIES OF HEMOLYTIC STREPTOCOCCI IN SCARLET FEVER. R. TUNNICLIFF, J. Infect. Dis. **49**:357, 1931.

During the course of scarlet fever, especially during convalescence and in complications, atypical hemolytic streptococci were isolated alone or associated with typical S. scarlatinae. They appeared to have dissociated from the typical, or "normal," slightly granular, conical scarlatinal colonies and differed from them

by forming on heated blood agar granular or smooth convex colonies and rough conical colonies with regular edges. The slightly granular and the rough conical colonies did not change chocolate agar; the granular convex colonies turned it green, while the smooth convex colonies varied in this respect. By the opsonic test, the cocci from dissociated colonies were not opsonified like the group of streptococci specific for scarlet fever, but by subculturing they were reverted, both colonially and immunologically, to typical scarlatinal streptococci. AUTHOR'S SUMMARY.

THE EFFECT OF HEAT, STORAGE AND CHLORINATION ON THE TOXICITY OF STAPHYLOCOCCUS FILTRATES. E. O. JORDAN, G. M. DACK and O. WOOLPERT, J. Prev. Med. **5**:383, 1931.

The toxic substance present in staphylococcus filtrates causing gastro-intestinal derangement is not completely destroyed by exposure for thirty minutes to the temperature of boiling water. Some diminution in toxic power may, however, possibly be caused by heating, even at temperatures below 100 C. Similarly, the toxic quality does not disappear after storage at a low temperature for as long as sixty-seven days, but is perhaps somewhat weakened. Contact for three minutes with a rather strong dose of chlorine did not destroy the toxic quality.

AUTHORS' SUMMARY.

A CASE OF FOOD POISONING APPARENTLY DUE TO STAPHYLOCOCCUS. E. O. JORDAN and J. R. HALL, J. Prev. Med. **5**:387, 1931.

An outbreak of food poisoning from eating chicken gravy was apparently caused by the products of growth of a stayphylococcus, which in pure culture formed substances that, when swallowed by human volunteers, reproduced the typical picture of the original outbreak.

AUTHORS' SUMMARY.

GLANDULAR FEVER—THE PROTOZOAL NATURE OF THE EXPERIMENTAL DISEASE. J. O. W. BLAND, Brit. J. Exper. Path. **12**:311, 1931.

The disease of rabbits, previously called "experimental glandular fever," which followed the inoculation of blood from two patients with glandular fever is caused by a protozoan of the genus Toxoplasma. This protozoan closely resembles T. cuniculi and is immunologically identical with it. The disease it causes is indistinguishable from that produced by T. cuniculi. The protozoan differs from ordinary T. cuniculi in its greater virulence for rabbits and in its power to infect monkeys, in which it produces a disease much like human glandular fever. No similar disease has been produced in rabbits of the same stock by control inoculations with normal rabbit or normal human blood or with blood from febrile people. The evidence suggests that human glandular fever may be caused by the protozoan described, but this requires confirmation.

AUTHOR'S SUMMARY.

THE RESISTANCE TO HEAT AND DISINFECTANTS OF PROTEIN-FREE ELUATES OF A BACTERIOPHAGE AND FOWL-POX VIRUS. I. J. KLIGLER and L. OLITZKI, Brit. J. Exper. Path. **12**:393, 1931.

Data are presented showing that the effect of heat and, more particularly, that of various disinfectants on a bacteriophage and fowl-pox virus, is weakened by the presence of protein. There is a general similarity in the reaction of the pure fowl-pox virus and bacteriophage to disinfectants, but there are differences in their relative susceptibility to one or another antiseptic. Both pure virus and pure bacteriophage are less sensitive to the action of phenol and ether than to any of the other reagents used.

AUTHORS' SUMMARY.

HEMATOGENOUS TUBERCULOSIS OF THE TONSILS. C. KRAUSPE, Verhandl. d. deutsch. path. Gesellsch. **26**:278, 1931.

Except in the first stages, it cannot be decided from histologic examination, whether in a given case tuberculosis of the tonsils is due to hematogenous or to oral infection. The problem was studied on rabbits after oral and intravenous injection of bovine tubercle bacilli. In both instances, the tubercles were found in the lymphoid tissue of the tonsils, especially in the area near the lymph follicles. In children with tonsillar tuberculosis, hematogenous tubercles are frequently found in the bones of the base of the skull. This fact suggests that tuberculosis of the tonsils also is most often due to a hematogenous infection.

C. ALEXANDER HELLWIG.

EXPERIMENTS ON ACUTE LYMPHOCYTOSIS. G. WALLBACH, Verhandl. d. deutsch. path. Gesellsch. **26**:352, 1931.

Suspensions of proteus and colon bacilli were injected intravenously into rabbits. In a few minutes, marked leukopenia with relative lymphocytosis was found, which continued for the most part until the next day. In this stage, death occurred in several of the animals. The following day, sometimes earlier, the condition of the other rabbits improved, at which time increasing leukocytosis was demonstrated. When the injection of bacteria was repeated, leukopenia was less noticeable; after the third injection it was almost completely missed. Leukocytosis is, according to the author, evidence of active immunization and follows an initial stage of destruction of granulocytes.

C. ALEXANDER HELLWIG.

COMPARATIVE PATHOLOGY OF PARASITIC DISEASES OF THE BRAIN. L. SINGER, Verhandl. d. deutsch. path. Gesellsch. **26**:357, 1931.

Six cases of coenurosis were studied histologically. The larval stage of Taenia coenurus causes typical mesenchymal changes in the brain substance, which resembles the tissue reaction in echinococcic disease of the liver. The parasite was found lying in a necrotic area, surrounded by epithelioid cells in pallisade arrangement and giant cells. At the periphery, a wall of lymphocytes had formed with typical lymph follicles.

C. ALEXANDER HELLWIG.

THE CULTIVATION OF SPIROCHAETA PALLIDA. R. R. HÖLTZER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **72**:320, 1931.

The so-called "cotton" culture mediums of Wrublewski, consisting in the use of cotton instead of animal tissues for adsorption of oxygen, proved unsatisfactory for growing Spirochaeta pallida. However, addition of 10 per cent rabbit or sheep serum gave a luxurient growth, equal to the one obtained in broth containing pieces of liver. A layer of petrolatum on the surface to exclude oxygen was necessary for the growth.

I. DAVIDSOHN.

CHEMICAL CHANGES IN THE SERUM OF GUINEA-PIGS WITH EXPERIMENTAL TUBERCULOSIS. T. WOHLFEIL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **72**:387, 1931.

The tryptophane was elevated in direct proportion to the severity of the disease. The increase varied from 21 per cent in animals with only local glandular involvement to 94 per cent in animals with diffuse glandular and visceral changes. A number of animals infected with a strain of low virulence showed, on superinfection with a very virulent strain two months later, markedly increased resistance and considerable rise in serum tryptophane as compared with animals that did not present increased resistance to superinfection. Tyrosine, nonprotein nitrogen and aminonitrogen were within normal range.

I. DAVIDSOHN.

HASTENING EXPERIMENTAL TUBERCULOSIS IN THE GUINEA-PIG. ADOLF HOCHWALD and ERICH TAUB, Ztschr. f. Immunitätsforsch. u. exper. Therap. **72**:462, 1931.

The injection of virulent tubercle bacilli was followed in twenty-four hours with a series of ten daily injections of a typhoid vaccine. The specific tuberculous changes were farther advanced and more diffusely spread out in animals treated with the vaccine than in controls. The apparent hastening of the tuberculous process is brought about, in the author's opinion, by the nonspecific effect of the foreign protein and the specific action of the typhoid toxin on the reticulo-endothelial system, and to some extent also by the loss of appetite, the fever and the peritoneal irritation.

I. DAVIDSOHN.

TUBERCULOSIS OF THE BRONCHIAL LYMPH NODES IN ADULTS. T. HAUSMANN, Ztschr. f. Tuberk. **61**:218, 1931.

In contradistinction to the usual teaching it is claimed that tuberculosis of the lymph nodes in adults is not rare. It occurs rarely in the presence of progressive pulmonary tuberculosis, but frequently in the absence of pulmonary lesions. The disease is diagnosable essentially by percussion.

MAX PINNER.

MONOCYTOSIS IN PULMONARY TUBERCULOSIS. H. VOS, Ztschr. f. Tuberk. **61**:305, 1931.

Monocytosis is frequently found in tuberculosis of adults, more frequently when the disease is severe than when it is slight. In favorable cases, monocytosis is more frequently associated with lymphocytosis; in unfavorable cases, with lymphopenia and shifting to the left. According to Schilling, monocytosis in tuberculosis is in most cases not to be considered as a prognostically favorable sign. The appearance of monocytosis is prognostically ambivalent.

MAX PINNER.

THE DIAGNOSTIC SIGNIFICANCE OF FECAL EXAMINATION FOR TUBERCLE BACILLI. D. SZÜLE, Ztschr. f. Tuberk. **61**:422, 1931.

In intestinal tuberculosis, the bacilli are demonstrable in every case. In pulmonary tuberculosis, they are demonstrable only in open cases. Frequently bacilli are demonstrable in the stools when they are not demonstrable in the sputum.

MAX PINNER.

TUBERCLE BACILLI IN THE BLOOD OF TUBERCULOUS PATIENTS. M. HÜTTIG, Ztschr. f. Tuberk. **62**:35, 1931.

Only seven of fifty-nine patients with pulmonary tuberculosis, in most of them far advanced, gave blood cultures positive for tubercle bacilli according to Löwenstein's method.

MAX PINNER.

FURTHER STUDIES OF THE DISASTER IN LÜBECK. B. LANGE, Ztschr. f. Tuberk. **62**:335, 1931.

At the time when the first death occurred following the B C G vaccination in Lübeck, a strain marked B C G 143 was present in the Lübeck laboratory. Careful tests for virulence made it quite certain that this strain was not one of B C G but a strain of a human tubercle bacillus of very low virulence for guinea-pigs. So far all evidence points to the assumption that pure B C G is unlikely to undergo sudden increases in virulence. Statements to the contrary are based on experimental work which so far is lacking confirmation. Although other

workers were able to derive morphologically different colonies from B C G, Petroff's contention that the S form is virulent has not been confirmed. It is concluded again that disease and death were caused in the vaccinated children because a pathogenic tubercle bacillus other than B C G had been administered. Since in vaccines a tubercle bacillus of low virulence was demonstrated, and since a tubercle bacillus of similar behavior was isolated from the diseased children this statement seems well founded, especially since atypical strains of such low and labile virulence for guinea-pigs are quite rare.

MAX PINNER.

TUBERCULOSIS IN THE FIRST THREE YEARS OF LIFE. J. OSTENFELD and R. KJER-PETERSEN, Ztschr. f. Tuberk. **62**:369, 1931.

A total of 260 families in each of which at least one member had proved open tuberculosis, and which had been observed during the years from 1919 to 1925, were studied in regard to the fate of children born in these families. A total of 245 children were available for the study. Of these 245 children, 19, or 7.8 per cent, died. In 12, the cause of death was undoubtedly tuberculosis. This percentage is far below the 25 or 30 per cent which is claimed by Calmette. From 1908 to 1925, 295 children were known to the dispensaries in Copenhagen to have had a positive Pirquet test within the first three years of life. In 1930, 293 of these children could be traced. Of 53 children who had a positive reaction during the first year of life, 33 were alive after at least five years. Of 91 children with a positive Pirquet test in the second year of life, 77 were living, and of 149 children with a positive test in the third year of life, 134 were living. Of all children who had died, only 6 had died after the age of 5 and only one of these had died of tuberculosis. It is concluded that a positive Pirquet test even in the first two years of life does not necessarily indicate active disease.

MAX PINNER.

Immunology

IMMUNITY TO VACCINATION BY INJECTION OF TESTICULAR VACCINE VIRUS. S. P. KRAMER, J. Infect. Dis. **50**:119, 1932.

When rabbits were treated by repeated injections of testicular vaccine virus that had been filtered through a basic filter, they became immune to vaccinia. When virus of dermal origin was used, immunity did not follow.

AUTHOR'S SUMMARY.

EFFECT OF TYPHOID FEVER AND PREVIOUS TYPHOID VACCINATION ON ANTIBODY RESPONSE TO TYPHOID VACCINE. R. F. FEEMSTER, J. Infect. Dis. **50**:121, 1932.

Persons who have had typhoid fever or who have been inoculated with typhoid vaccine produce larger amounts of antibodies in response to subsequent inoculations with typhoid vaccine than those who have not had typhoid or have not been vaccinated against the disease. The routine injection of three doses of typhoid vaccine may not give as high a degree of immunity as has been believed. A second or third injection some weeks or months after the first would seem to be indicated. The Widal reaction is less reliable in the case of a person who has been vaccinated two or more times than in that of a person who has been vaccinated only once. The complement-fixation reaction is no more reliable than the agglutination reaction in diagnosing infection with the typhoid bacillus. The difficulties and uncertainties encountered in carrying out and interpreting bactericidal tests render the procedure unsatisfactory for the routine diagnosis of typhoid fever. The severity of the reaction to the injection of typhoid vaccine is in no way related to the immunity that is formed in response to the injection.

AUTHOR'S SUMMARY.

STAPHYLOCOCCUS AUREUS AGGLUTININS IN TUBERCULOUS EFFUSIONS. M. PINNER and M. VOLDRICH, J. Infect. Dis. **50**:143, 1932.

Tuberculous effusions invariably contain a factor that behaves serologically like an agglutinin specific for Staphylococcus aureus. The majority of nontuberculous effusions tested do not agglutinate S. aureus; neither does blood serum from tuberculous patients.

AUTHORS' SUMMARY.

PRECIPITINOGENIC ACTION OF MINUTE QUANTITIES OF OVALBUMIN. L. HEKTOEN and A. G. COLE, J. Infect. Dis. **50**:171, 1932.

Two rabbits were immunized against ovalbumin by injections of 0.000,047 Gm. and 0.000,27 Gm., respectively, of protein nitrogen, corresponding to approximately 0.000,29 Gm. and 0.001,7 Gm. of ovalbumin. The antiserums obtained gave precipitates with the homologous antigen in the dilution 1 : 100,000.

AUTHORS' SUMMARY.

ANTIGENIC PROPERTIES OF CARBOHYDRATE AND PROTEIN FRACTIONS OF MENINGOCOCCI. J. ZOZAYA and J. E. WOOD, J. Infect. Dis. **50**:177, 1932.

The polysaccharide and the "nucleoprotein" obtained from meningococci of the different types, gonococci and Micrococcus catarrhalis have similar immunologic properties and are not type-specific. The carbohydrate-precipitable substance in immune serums appears late (fourth month or more) in the immunization of animals. There is no parallelism between the agglutination test and the polysaccharide precipitin test.

AUTHORS' SUMMARY.

NATURAL IMMUNITY TO STAPHYLOCOCCAL TOXIN. L. M. BRYCE and F. M. BURNET, J. Path. & Bact. **35**:183, 1932.

The distribution of staphylococcic antitoxin in human serum according to age is similar to that obtaining for diphtheritic antitoxin. In about 50 per cent of infants, the blood at birth contains the same concentration of antitoxin as the mother's blood, but gross divergences are not uncommon and point to a considerable lag in the passage of antitoxin across the placenta. Natural staphylococcic antitoxin is frequently present in domestic rats, and evidence is presented which indicates that its occurrence is a true antibody response to contact with the specific antigen. In many persons, the Dick type of reaction to intradermal injection of staphylococcic filtrates is not a manifestation of the activity of staphylococcic toxin.

AUTHORS' SUMMARY.

CELLULAR IMMUNITY TO SNAKE VENOM AND STAPHYLOCOCCAL TOXIN. F. M. BURNET, C. H. KELLAWAY and F. E. WILLIAMS, J. Path. & Bact. **35**:193 and 199, 1932.

The perfused heart muscle of a rabbit immunized to cobra venom was less susceptible to the action of this venom than that of a normal rabbit. Adding small amounts of immune serum to the bath in which the normal isolated auricle was suspended caused it to behave with venom like the auricle from an immunized animal, suggesting that in the immunized animal there might be an accumulation of antibody in the tissue spaces not readily removable by perfusion. In immunization of animals with staphylococcic toxin it was found that there is an accumulation of antibody in the tissue spaces which cannot be removed by perfusion. The results throw doubt on the value of the perfusion experiment for the demonstration of antitoxic cellular immunity.

IMMUNE SERA AGAINST FOWL-TUMOUR VIRUSES. C. H. ANDREWES, J. Path. & Bact. **35**:243, 1932.

The development of antibodies to the Rous virus may be followed in the serums of fowls bearing either of two slow-growing fibrosarcomas (MH1 and CT10). Potent antiserums were not found in fowls that had borne tumors for less than five months. Neutralization of Rous virus by immune serum is more complete when the mixtures are incubated at 37 C. It is possible to recover virus from an inactive serum-virus mixture, provided that serum and virus have not been long in contact. Resemblances between the serums under discussion and other antiviral serums are pointed out.

AUTHOR'S SUMMARY.

STAPHYLOCOCCAL TOXIN AND ANTITOXIN. H. J. PARISH and W. H. M. CLARK, J. Path. & Bact. **35**:251, 1932.

Staphylococcic septicemia is comparatively rare and rapidly fatal. Staphylococcic toxin can be prepared in an atmosphere containing 25 per cent carbon dioxide in Parker's 4 per cent peptone buffered broth, on Burnet's 0.8 per cent agar plates, and in Winchester bottles containing 0.8 per cent agar to which Parker's broth is added after forty-eight hours' growth. The toxin kills rabbits, guinea-pigs and mice when given intravenously, produces cutaneous lesions in rabbits and guinea-pigs, and is actively hemolytic. It is rapidly converted into toxoid by formaldehyde. Horse antitoxin can be prepared that protects rabbits against a lethal dose of toxin injected intravenously. It is best titrated by injecting mixtures of toxin and antitoxin intracutaneously into guinea-pigs, and by testing its antihemolytic power. The antitoxin can be concentrated by ammonium sulphate precipitation. When given intravenously to rabbits prior to a certainly fatal dose of virulent living culture, it prolongs and in some cases saves life. The favorable results that have attended its use in a small number of patients justify more extensive clinical trial.

AUTHORS' SUMMARY.

THE EFFECT OF LEAD ON THE PRODUCTION OF IMMUNE SUBSTANCES. F. W. BICKERT, Arch. f. Hyg. **106**:271, 1931.

Rabbits given a series of daily subcutaneous injections of 1 cc. of a 5 per cent solution of lead acetate appear to produce a greater yield of antisheep hemolysin than rabbits not so treated. The maximum titer is reached more slowly in the lead-treated animals than in the controls. Preliminary injections of lead salts appear to favor production of agglutinin and antitoxin, also, but to inhibit the production of precipitin.

ARTHUR LOCKE.

THE EFFECT OF BENZENE AND TURPENTINE ON THE PRODUCTION OF IMMUNE SUBSTANCES. F. W. BICKERT, Arch. f. Hyg. **107**:1, 1931.

Rabbits given intravenous injections of washed sheep erythrocytes produce a diminished and retarded yield of antisheep hemolysin when they have previously received subcutaneous injections of benzene. Subcutaneous injections of turpentine appear to favor the production of hemolysin and leukocytes.

ARTHUR LOCKE.

THE LIPOLYTIC CONTENT IN SPUTUM IN PULMONARY TUBERCULOSIS. S. PUDER, Beitr. z. Klin. d. Tuberk. **79**:98, 1931.

For measuring the amount of lipase in sputum the titrimetric method is best. One hundred sputums obtained in cases of tuberculosis were examined, and it was found that in all cases the amount of lipase was minimal, but that it increased somewhat in severe cases. Since in severe cases the lipase content is decreased in serum it is assumed that the decrease is due to excretion of the lipase through the sputum.

MAX PINNER.

GROUP-SPECIFIC REACTIONS OF THE STROMA OF RED CORPUSCLES. F. OTTENSOOSER and ST. ZURUKZOGLU, Klin. Wchnschr. **17**:719, 1932.

The group reactions of the stroma, according to Dold and Rosenberg, corresponded in 85 per cent of instances with the results of the usual tests for blood groups. An improved modification of the method, as well as a new method, is described. As a rule, the stroma gives a lower titer of agglutination than the corpuscles, but on preservation the stroma remains, in most instances, more agglutinable than the corpuscles.

FROM THE AUTHORS' SUMMARY.

IMMUNITY IN RABIES. E. LOEFFLER and F. SCHWEINBURG, Virchows Arch. f. path. Anat. **279**:181, 1930.

The results of the experiments described failed to support the older theory of the formation of antibody within the brain in rabies. Immunity could not be produced by intracerebral injection of fixed virus, but followed intramuscular, intraperitoneal and subcutaneous injection. The findings indicate that the formation of antibody occurs, not within the brain, but in the other tissues of the body.

W. SAPHIR.

ACTIVE IMMUNIZATION AGAINST DIPHTHERIA. M. P. ISABOLINSKI, B. P. KARPATSCHEWSKAJA and W. F. TOWJANSKAJA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:27, 1931.

Active immunization with Ramon's anatoxin lowered the morbidity from seven to ten times in 4,185 children. The local and general reactions were of no significance. The percutaneous method of Loewenstein is strongly recommended.

I. DAVIDSOHN.

PRODUCTION OF ANTIBODIES AGAINST ORGANS OF THE HOMOLOGOUS SPECIES. S. SCHILLING-SIENGALEWICZ and W. BIELOSZABSKI, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:31, 1931.

The cold precipitation technic of Kumagai and Yanabashi was checked and found correct. In addition to the already established serologic relationship between the kidney and the liver, such a relationship was found between the latter organ and the thyroid gland.

I. DAVIDSOHN.

THE BLOOD ANTIGENS IN BACILLUS PARATYPHOSUS B AND BACILLUS DYSENTERIAE SHIGA. M. EISLER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:37, 1931.

B. paratyphosus B and B. dysenteriae Shiga contain heterophilic (Forssman) antigen. Only the homologous bacteria are able to absorb the antisheep hemolysin produced by them, but they are unable to bind the hemolysins produced by sheep red cells and by guinea-pig tissues. The heterophilic antigen in Shiga bacilli is contained in their carbohydrate fraction; that of paratyphoid B bacilli, in their watery and alcoholic extracts, but not in their carbohydrate fraction. The carbohydrate fraction of Shiga bacilli contains also a human blood antigen (M-antigen) showing no group specificity; paratyphoid B bacilli contain an alcohol-soluble antigenic substance related to the human A-receptor.

I. DAVIDSOHN.

THE TWO TYPES OF THE BLOOD GROUP QUALITY A IN MAN. M. AKUNE, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:75, 1931.

The A-large and the A-small type of the blood group A can be differentiated with the help of hetero-antibodies as well as with iso-antibodies. The differences are preserved in aqueous and alcoholic extracts of the red blood cells and are

present in the blood serum, saliva and urine. Red blood cells of type A-large more frequently produce group-specific antiserums in the rabbit than those of type A-small; specific antiserums for the A-small red blood cells could not be obtained.

I. DAVIDSOHN.

SUBSTANCES STIMULATING PERISTALSIS AND LOWERING BLOOD PRESSURE IN ORGANS OF SENSITIZED AND SHOCKED DOGS. K. WATANABE, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:149, 1931.

An increase of the substances mentioned in the title was observed in livers of sensitized animals followed by a marked drop during anaphylactic shock. No such changes were found in the other organs, except the spleen, which showed a marked increase both in sensitized and in shocked dogs. I. DAVIDSOHN.

AGGLUTINATION OF SHEEP RED BLOOD CELLS BY HUMAN SERUM IN COMPLEMENT-FIXATION TESTS. OSCAR FISCHER, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:154, 1931.

A marked agglutination of sheep red blood cells was observed in a positive complement-fixation test for gonorrhea. Agglutination was not present and hemolysis took place when syphilitic antigen was employed. In the latter case free complement was available. Agglutination occurs only when the complement is fixed. Agglutinins for sheep red cells do not interfere with hemolysis in the presence of complement.

I. DAVIDSOHN.

THE CONCENTRATION OF ANATOXIN. W. M. KULIKOW and A. W. BEILINSSON, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:178, 1931.

The precipitation of proteins and of related nitrogenous substances was accomplished with hydrochloric acid in the presence of certain anticoagulants. Four fifths of the nitrogenous substances and all the solids were removed in this manner. The anatoxin sediment was easily soluble and produced no local reactions in the guinea-pig.

I. DAVIDSOHN.

THE RÔLE OF THE RETICULO-ENDOTHELIAL SYSTEM IN IMMUNITY. B. P. KARPATSCHEWSKAJA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:190, 1931.

Trypan red, india ink and phenylhydrazine were used for the blocking of the reticulo-endothelial cells before and after immunization of guinea-pigs with diphtheria anatoxin. The antibody response was adversely affected, particularly when india ink was used.

I. DAVIDSOHN.

IMMUNOLOGIC STUDIES IN PNEUMOCOCCIC INFECTIONS. M. GUNDEL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:193, 1932.

Serums of 126 persons of various ages were examined for type-specific antipneumococcic protecting substances and agglutinins. They included healthy persons and such as were suffering from various pneumococcic infections. In 5 of 15 normal adults with no history of previous pneumococcic infection, considerable quantities of such substances were found. They were absent in 12 healthy newborn infants and young children. In patients suffering from bronchopneumonia and from pneumococcic infections of the upper respiratory tract, type-specific protecting substances and agglutinins could not be detected. They were found in large quantities in persons with lobar pneumonia and postpneumonic empyema due to type I and type II pneumococci. They appeared infrequently before the crisis, but reached their maximum during convalescence. In type III infections,

antibodies were rarely produced. Children till the end of the second year are poor producers of antibodies. In otitis media and in peritonitis due to pneumococci, the development of protecting substances and less frequently of agglutinins was observed, while the serums of type I and type II pneumococcus carriers were devoid of protecting and agglutinating qualities. The use of convalescent serums for therapeutic purposes is advocated. Slight development of antibodies was observed in children about 1 year old and older, following vaccination with killed pneumococci.

I. DAVIDSOHN.

THE MECHANISM OF COMPLEMENT FIXATION AND OF THE PRECIPITATION REACTION. D. P. BOROWSKAJA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:239, 1932.

In the Meinicke reaction and a slightly modified citochol reaction, the complement of the active serum is not affected by the flocculation, which takes place in a hypertonic medium. The natural antisheep hemolysin was also found in the supernatant fluid after the precipitate was removed by centrifugation. It is concluded that flocculation and complement fixation do not always run parallel, at least not in a hypertonic medium.

I. DAVIDSOHN.

SEROLOGIC DEMONSTRATION OF BRAIN ANTIGEN IN THE BLOOD. F. PLAUT and H. RUDY, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:242, 1932.

Serum of rabbits fed with brain substance or given subcutaneous injections of it did not react in the complement-fixation test with brain antiserum. Reaction occurred when large quantities of the antigen were injected intravenously. The lack of reaction is due to the masking effect of serum lipoids on the injected antigen, as shown in vitro. The acetone-insoluble fraction of the serum lipoids seems to be the factor responsible for the masking. The antigenic effect of the brain lipoids reappeared following removal of the acetone-insoluble fraction.

I. DAVIDSOHN.

GLUCOSIDES AND VACCINATION WITH VIRULENT BACTERIA. K. HRUŠKA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:256, 1932.

Virulent anthrax bacilli and spores suspended in tissue irritants, saponin and digitonin, can be injected subcutaneously into rabbits and large animals with resulting immunity. No such protection is obtained in guinea-pigs. The local necrosis produced by the irritants prevents the generalization of the infection. The virulence of the bacilli kept for a long time in the solutions of saponin and digitonin is not affected. Proper concentration and large doses of the irritants are essential, as in case of too small quantities the insufficient local reaction is unable to stop the spread of the infection. The glucoside of the chestnut (Rosskastanie) has a local necrotizing effect, but lacks the protecting property.

I. DAVIDSOHN.

COMPLEMENT FIXATION WITH SOLUTIONS OF RED BLOOD CELLS. E. BRUNIUS, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:293, 1932.

The solutions were employed for the demonstration of the Forssman antigen in the red cells of the guinea-pig with equally good results as alcoholic extracts. Heating to 100 C. destroyed their complement-fixing ability, which reappeared in alcoholic extracts of the same heated solutions. Species and group-specific antigens were retained in such solutions of red cells of man and pig. The group-specific qualities could be better demonstrated with the help of the complement-fixation test, in which the dissolved red blood cells were used as an antigen, than when the agglutination with native red blood cells was employed. However, alcoholic extracts are still better for demonstration of group specificity.

I. DAVIDSOHN.

INACTIVATION OF AGGLUTININS BY DIGESTIVE SECRETIONS. A. A. SCHMIDT and KLARA TULJTSCHINSKAJA, Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:312, 1932.

Agglutinins for Bacillus typhosus were destroyed by active, and to a lesser extent by heated gastric juice. The hydrochloric acid is probably an important factor, as it effects the same result by itself. Active pancreatic secretion had a weaker effect, while active intestinal ferments did not harm agglutinins.

I. DAVIDSOHN.

Tumors

THE METABOLISM OF TUMOR CELLS. G. SCHMIDT, Frankfurt. Ztschr. f. Path. **41**:393, 1931.

This article deals with the catabolic and anabolic properties of tumor cells, especially with the process of glycolysis. The more important literature on the subject is reviewed, but original observations are not given. The author points out that it still remains a matter of further research to study quantitatively synthetic processes of tumor cells.

O. SAPHIR.

CARCINOMA OF THE PROSTATE GLAND AND CRYPTORCHISM IN A DOG. C. KRAUSE, Frankfurt. Ztschr. f. Path. **41**:405, 1931.

In an 8 year old dog in which the left testis had been removed six years previously, the right testis and epididymis were found in the right iliac region. The surface of this testis appeared irregular, granular and hemorrhagic. A few dark red areas of softening were found in the center. The prostate gland was almost completely transformed into two vesicle-like structures. Parts of the walls of these vesicles were necrotic, crossed by firmer bridges of tissue recognized grossly as tumor. The urethra contained many undermined ulcers in addition to circumscribed thickenings. In the histologic sections of the testis, accumulations of cells were arranged in the form of alveolus-like structures, some of which were completely filled with tumor cells. Some of these cells were large, with vesicular nuclei and much cytoplasm; others small, resembling lymphocytes; a few polygonal, containing round nuclei. There were only few mitotic figures. The prostate gland microscopically was composed of tubular structures, masses of epithelial cells and cystlike structures, in addition to many inflammatory cells.

The author, who states that the finer histologic details will be given in another paper, concludes that the tumor of the testis is neither characteristic of carcinoma or sarcoma, but should be classified as a precancerous condition. The tumor of the prostate gland is classified as carcinoma.

O. SAPHIR.

GROUP-SPECIFIC AND HETEROGENEOUS STRUCTURES IN THE LIPOID ANTIGEN OF MALIGNANT TUMORS. H. LEHMANN-FACIUS, Frankfurt. Ztschr. f. Path. **41**:521, 1931.

This detailed article deals mainly with problems of method in the study of specificity of immune serum in cases of malignant tumors.

O. SAPHIR.

PLEXIFORM NEUROMA WITH PACINIAN CORPUSCLES. M. BRÖGLI, Frankfurt. Ztschr. f. Path. **41**:595, 1931.

Such a tumor is described in the lumbar region of a 16 year old youth. The author believes that this is the first case reported of a neuroma in which typical pacinian corpuscles were found.

O. SAPHIR.

EFFECT OF ROENTGEN RAYS ON THE METABOLISM OF JENSEN SARCOMA. A. LÖW-BEER and M. REISS, Strahlentherapie **42**:157, 1931.

The metabolism of slices of irradiated Jensen sarcoma was examined with the Warburg method. It was found that there was a shift from the oxidative to the fermentative type of metabolism. In normal tissues this shift occurs later and after higher doses of x-rays. This difference in response is attributed to differences in radiosensitivity.

WILHELM C. HUEPER.

VARIATIONS IN THE LACTIC ACID CONTENT OF THE BLOOD AFTER ROENTGEN TREATMENT. J. HEEREN and R. HUMMEL, Strahlentherapie **42**:784, 1931.

Roentgen irradiation of the tumor resulted in an increase in the lactic acid of blood removed from six to eight minutes after treatment (an increase of from 22.9 to 102 per cent) in sixteen of nineteen cases. After irradiation of the thigh muscles of the same patients (six), a definite decrease in the lactic acid (of from 7.8 to 44 per cent) was seen. Similar results were obtained in noncarcinomatous persons (decrease in lactic acid of from 10 to 15.6 per cent). The location of the tumor had no effect on the result obtained. Irradiation of the hypochondric and splenic region did not change these results. The increase in lactic acid of the blood in patients with cancer after irradiation of the tumor is therefore not an indirect effect.

WILHELM C. HUEPER.

THE PATHOGENESIS OF CARCINOIDS. F. FEYRTER, Verhandl. d. deutsch. path. Gesellsch. **26**:286, 1931.

The carcinoids of the human intestinal tract are not congenital malformations, but true new growths. Fifty-one of seventy-five carcinoids studied by the author were located in the jejunum and ileum. In this portion of the intestines, the carcinoid cells are related to the so-called yellow cells of Schmidt, while those in the duodenum and stomach must have another origin, since they are not argentaffin and not chromaffin. They resemble more the cells in Langerhans' islands and the peculiar clear cells that are normally found in the surroundings of the excretory duct of the pancreas.

C. ALEXANDER HELLWIG.

THE PATHOGENESIS OF ADIPOSITY IN CARCINOMA. E. MATHIAS, Verhandl. d. deutsch. pathol. Gesellsch. **26**:289, 1931.

Some patients with carcinoma show, in spite of long duration of the disease, a high grade of adiposity. Wohlwill found that such patients may have metastases of the cancer in the pituitary gland or its surroundings. Considering only patients in whom the subcutaneous tissue of the anterior abdominal wall had a thickness of from 6 to 8 cm., Mathias found metastatic carcinoma in the hypophysis in a few. In the majority of his cases, however, this explanation did not hold. The author believes that the continuous loss of blood in carcinomas of the stomach, rectum and cervix or in multiple metastases in the bone marrow is responsible for severe anemia, and that the resulting anoxemia is the causative factor of adiposity in these cases.

C. ALEXANDER HELLWIG.

INFLUENCE OF LOCAL CIRCULATORY DISTURBANCE AND ANEMIA ON THE GROWTH OF TRANSPLANTED TUMORS. B. MORPURGO, Verhandl. d. deutsch. path. Gesellsch. **26**:292, 1931.

An osteoid sarcoma was implanted into one kidney of each of a number of white rats. In one group of these animals, active hyperemia of the kidney containing the engrafted tumor was produced by removing the other kidney. In the second group, the blood vessels of the kidney containing the tumor were ligated, to produce permanent anemia. In both experimental series the graft survived, but

in the hyperemic kidney the transplanted tumor reached a much larger size than in controls, and in the anemic kidney the rate of growth was much slower. To study the influence of general anemia, osteoid sarcoma was implanted under the skin of white rats and severe anemia produced by frequent bleeding of the animals. In the anemic rats the rate of growth of the transplanted tumor was not less active than in normal animals, and also the duration of life was not shortened by the general anemia.

C. ALEXANDER HELLWIG.

LIPOID CONTENT OF MALIGNANT TUMORS. R. BIERICH, A. DETZEL and A. LANG, Ztschr. f. physiol. Chem. **200**:157, 1931.

Malignant tumors have relatively high phospholipoid and cholesterol ester. In malignant tumors, the quotient phospholipoid: cholesterol ester is small, while in normal tissues it is high. Normal tissues contain relatively small amounts of both substances. There was no increase or decrease in the lipoid of the tissues surrounding the tumor. Benign tumors have lower cholesterol ester and phospholipoid than malignant ones. The quotient is, however, the same as in malignant tumors.

WILHELM C. HUEPER.

Medicolegal Pathology

CHANGES OF THE HYPOPHYSIS AND THE CHIASM IN SEVERE TRAUMA. C. F. PORTA, Beitr. z. gerichtl. Med. **11**:83, 1931.

This study is based on observations of thirty-five cases. In thirty-two, the skull was fractured, nine of these revealing complete destruction of the base of the skull. In three instances, there were only contusions of the brain. The author concludes that rupture of the hypophysis occurs only in cases in which there is complete destruction of the skull. Such a condition is incompatible with life. Gross and microscopic changes of the hypophysis due to trauma are, in general, rare. Hemorrhages and contusions, which sometimes occur, are located in the center of the anterior lobe. Changes in the chiasm resulting from trauma are only occasionally encountered. A relation between trauma and clinical symptoms referable to lesions of the hypophysis should be regarded only with the greatest reserve. Such a relation might exist in cases of very severe trauma.

O. SAPHIR.

STRUCTURAL CHANGES IN CHRONIC POISONING WITH ARSINE. K. BEÖTHY, Beitr. z. gerichtl. Med. **11**:97, 1931.

Two dogs were fed with from 6.305 to 6.398 mg. of arsine per kilogram of body weight over a period of three months. Most commonly, recent and old hemorrhages were found in the thymus, stomach, small and large intestines, spleen, kidneys, lungs, lymph nodes and mucosa of the urinary bladder. None were found in the subendocardial spaces. Peyer's patches and solitary follicles, which contained only mature lymphocytes, were edematous and enlarged. Macrophages were found in the spleen. The intestinal tract revealed the most severe inflammatory changes.

O. SAPHIR.

LARYNGEAL WOUNDS IN THE NEW-BORN INFANT DUE TO ATTEMPTS AT REVIVAL. A. FELLER, Beitr. z. gerichtl. Med. **11**:150, 1931.

In two instances, endolaryngeal wounds in asphyxiated new-born infants were produced by the introduction of tracheal catheters for the purposes of revival. In each instance the trauma was in the anterior wall of the larynx corresponding to the ligamentum conicum. The autopsy also disclosed subcutaneous emphysema, which extended into the mediastinum. One of the infants revealed, in addition, bilateral pneumothorax.

O. SAPHIR.

TWO RUPTURES OF THE LEFT VENTRICLE FROM INTERNAL CAUSES. L. BREITENECKER, Beitr. z. gerichtl. Med. **11**:181, 1931.

In one instance, two areas of myomalacia were found in the anterior wall of the left ventricle, both of which had ruptured, with resulting hemopericardium. There was marked arteriosclerosis involving the main branches of the left coronary artery. In the second instance, two points of rupture were demonstrated in one large area of the myocardium situated in the posterior wall of the left ventricle, likewise with hemopericardium. The descending branch of the right coronary artery was occluded by calcified plaques.

O. SAPHIR.

SPONTANEOUS DIFFUSE SUBARACHNOIDAL HEMORRHAGE. O. HESS, Deutsche med. Wchnschr. **58**:45, 1932.

It is claimed that diffuse subarachnoidal hemorrhage may occur spontaneously in healthy persons, especially young persons, with a normal vascular system. Such hemorrhage may be due to vasoneurotic disturbances of the leptomeningeal vessels, but in every case most careful search should be made for structural vascular changes and particularly for basal aneurysm.

FINDINGS OF DIAGNOSTIC VALUE ON BURNT AND CHARRED HUMAN BODIES. HERMANN MERKEL, Deutsche Ztschr. f. d. ges. gerichtl. Med. **18**:232, 1931.

Destruction of a human body by fire offers great difficulties in correct interpretation. Roentgenologic examinations of the collected parts of the débris may disclose presence of bone fragments. For the differentiation between human and animal bones, the biologic tests, either on the still attached tiny particles of flesh or with ground bone, may be successfully employed. The teeth may be of valuable assistance in identification. While amalgam and gold dental work may be destroyed by fire, porcelain material resists the highest temperatures, as much as from 700 to 900 C., and has been found even in the ashes of cremated bodies. The most important question to decide is: Was the person burnt while alive or dead? Evidence of carbon monoxide in the internal organs, as in the blood of the cardiac cavities and in the aorta, liver, abdominal veins, etc., is conclusive evidence of existence of life during the fire, while presence of carbon monoxide in the blood vessels or tissues beneath the surface of the body alone may occur after death if the body was exposed to this gas in an enclosed environment. Absence of carbon monoxide in the organs does not exclude the possibility that the person was alive at the time of the exposure to fire, since psychic shock may lead to a sudden collapse, or aspiration of vomitus in the initial stages of carbon monoxide poisoning may cause death from asphyxiation. Presence of soot or smudging in the bronchial tree may serve as an additional diagnostic sign. An epidural hemorrhage or hematoma may develop in dead bodies under the influence of intense fire, and, if the head was fractured or crushed by falling débris after death, one may be deceived into assuming a vital injury. Stab wounds usually appear considerably smaller, owing to shrinkage of the drying skin. Destructions of the extremities are not uncommonly observed and then charred stumps of long bones may project freely. Extensive fracturing of bones that possess a well developed marrow space occurs on account of an explosive action. Fracture of the thyroid cartilage may be an artefact of the fire and should be carefully evaluated as a sign of strangulation. Microscopic evidence of embolism of liver cells or brain tissue is undoubtedly a positive proof of intravital injuries. Presence of minute fat globules in the territory of the pulmonary artery was found by several observers and is interpreted as evidence of vital injuries prior to the destruction of the body by fire.

E. L. MILOSLAVICH.

IDENTIFICATION OF BURNT BODIES BY MEANS OF THE TEETH. K. BOEHMER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **18**:250, 1931.

Identification of a human body destroyed by fire is a most difficult task. However, it is often achieved by an accurate examination of the teeth with the characteristic findings thereon, since they resist extreme temperatures and processes of decomposition. A pertinent case is presented in minute detail, and the mode of procedure is illustrated. A caoutchouc (india rubber) plate containing an upper incisor was not destroyed by the fire, contrary to the opinion of Gebhardt, who assumed that this material succumbs readily. Prolonged exposure to high degrees of temperature may cause calcination of the teeth, particularly of the exposed incisors, but they do not fall apart; their structure remains still evident. Pieces of enamel may occasionally crack and break off, the edges of the broken-off area appearing angular and sharp in contrast to a carious area, which is more or less round and smooth-edged. All the teeth in every decade of life present the same behavior toward fire; the front teeth, however, being exposed and anatomically unprotected, are most readily attacked. Teeth on which dental work has been done are more resistant than others. Experiments on teeth by exposing them to fire disclosed some interesting data. Fillings of silicate cement and crown cement became more hard, and consequently these teeth appeared less cracked than the unrepaired ones. Silver amalgam withstood only lower temperatures, while gold-platinum amalgam resisted higher degrees of and longer exposures to fire. Copper amalgam showed the strongest resistance.

E. L. MILOSLAVICH.

DEATH BY EXPOSURE TO COLD. KARL MEIXNER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **18**:270, 1931.

Clinical observations made on a 68 year old man who was found in the Tyrolian Alps, frozen but still alive, are interestingly presented. Respiration was noticeable, but the body was pulseless and apparently without heart beat. The lower part of the legs and both feet were hard and completely frozen. After energetic attempts at revival, the man, as in a daze, mumbled a few words and died fifteen hours later. Autopsy showed a slight lysis of the blood, but otherwise the erythrocytes were intact. Microscopic examination of the frozen legs disclosed the most marked changes in the epidermis and subcutaneous blood vessels. The external layers of the epidermis down to the stratum lucidum appeared as if exfoliated. The germinative layer presented greatly changed nuclei, which were either shrunken and then surrounded by a light, ringlike space, or swollen and in stage of lysis. The blood vessels were contracted or collapsed, and their endothelial elements were present sparsely and without nuclei. Fibrin was not evident. The striated muscles presented a pronounced crumbling of their protoplasm in places, while in the adjoining portions the striation was present. The cells of the intramuscular fat tissue contained needle-like, yellowish-brown crystals. They apparently developed on account of action of dissolved coloring matter of the blood and muscle tissue on the fat substance. The success of resuscitation depends on the length of time of exposure to cold, since the greatly lowered circulation of the blood produces irreparable damages in the vital centers. Meixner cites the case of a 25 year old nurse who, in a suicidal attempt, ingested morphine and veronal and was found, cold and apparently dead, in a park. A physician pronounced her dead, but the next morning, after she had lain for fifteen hours in a coffin at the morgue, it was discovered that she was alive. Resuscitation was successful, and her life was saved. These and similar cases are an earnest warning against attesting a body dead, in instances of exposure to cold, without a conscientious and thorough examination, as one may be confronted with only an apparent death.

E. L. MILOSLAVICH.

TEARING OF THE MUCOUS MEMBRANE OF THE STOMACH IN DEATH BY DROWNING. ERICH FRITZ, Deutsche Ztschr. f. d. ges. gerichtl. Med. **18**:285, 1931.

In four cases of death due to drowning, linear lacerations of the gastric mucous surface along the lesser curvature and cardia were found, but there was no evidence of external violence. In one instance, the tears were located mainly around the cardiac opening, and in two the proximal third of the lesser curvature was involved. In the fourth case, which showed a tearing also of the serosal covering, the distal third was affected. In all the cases, punctate hemorrhages were present, not only within the torn tissues, but also in the immediate vicinity of these tissues, indicating that the tearing happened during life. A traumatic rupture of the stomach may be the result of direct external violence, and then it occurs chiefly close to the pylorus or on the anterior wall of the stomach. In none of the thirteen cases described by Rehn was the lesser curvature found involved. The mechanism of the tearing during drowning is explained by the increase of intragastric pressure. Vomiting, which is frequently observed in drowning, produces a contraction of the pyloric portion of the stomach, while the cardiac area undergoes a partial distention, thus leading to a tearing of the mucous surface around the cardiac mouth.

E. L. MILOSLAVICH.

AN UNUSUAL, NOT PREVIOUSLY REPORTED CADAVERIC CHANGE. PHILLIP SCHNEIDER, Deutsche Ztschr. f. d. ges. gerichtl. Med. **18**:325, 1931.

Changes of the eyeballs occur soon after death and consist of an opaque appearance and drying up of the external surface, and loss of tension, causing collapse of the bulbi. If the vitreous body liquefies, the eye may appear as a flabby sac. In a woman, aged 48, who died of carcinoma of the uterus with peritoneal metastases, both eyeballs showed, a few hours after death, complete liquefaction and had disappeared altogether. The orbital cavities were filled with a dark-colored, bloody fluid containing gas bubbles, and this fluid material was running down the cheeks. Culturally, Bacillus mesentericus, Bacterium coli and hemolytic streptococci were obtained from the orbital contents. This peculiar cadaveric phenomenon is explained as the effect of terminal bacteremia causing putrefactive destruction of both eyes.

E. L. MILOSLAVICH.

OCCUPATIONAL PNEUMONOCONIOSIS IN AN EXHUMED BODY. E. ŻIEMKE, Deutsche Ztschr. f. d. ges. gerichtl. Med. **18**:330, 1931.

Autopsy on an unembalmed body ten weeks after interment clearly demonstrated pneumonoconiotic changes. The roentgenographic studies of the lungs during life, made by experienced specialists, were not conclusive as to presence or absence of coniotic lesions, hence the value of postmortem examination in such instances is emphasized. The microscopic changes are presented in detail, and the question of a macronodular type of silicosis in its relation to pulmonary tuberculosis is mentioned.

E. L. MILOSLAVICH.

Society Transactions

NEW YORK PATHOLOGICAL SOCIETY

Regular Meeting, May 26, 1932

CHARLES T. OLCOTT, *Vice-President, in the Chair*

ECHINOCOCCUS CYST FORMING THE CONTENT OF VENTRAL HERNIA. LAWRENCE H. COTTER.

A laborer, 30 years old, born near Naples, had always worked in a vineyard until coming to this country about ten years ago. About eight years ago he noticed a ventral hernia, which soon reached its maximum size. This hernia caused him little trouble and was easily reducible. He kept it in place by wearing high-waisted trousers and at times also wore a bandage around the abdomen. On the day of his admission to the hospital, the mass became very painful, and he was unable to reduce it as he had previously.

Physical examination showed a man suffering from acute pain. There was a tumor mass in the midline of the abdomen, above the umbilicus, which was tender on pressure and not reducible. It was about 10 cm. in diameter.

At operation, a cystic mass about 10 cm. in diameter was found to be the content of the hernia. The cyst was attached to the left lobe of the liver. It was filled with clear fluid, and contained five daughter cysts, each about 2 cm. in diameter. The daughter cysts were free in the fluid of the parent cyst. The fluid contained numerous hooklets and scolices.

PATHOLOGY OF HEMOLYTIC JAUNDICE. WILLIAM P. THOMPSON (by invitation).

A study of the clinical and pathologic findings in thirty cases of hemolytic jaundice is reported. Of these thirty cases, twenty-six presented the usual clinical findings associated with the congenital type of this disease, although in many of the twenty-six cases evidence of the congenital or familial nature was lacking.

The spleens in these twenty-six cases presented a uniform and characteristic appearance. They weighed from 1,000 to 1,500 Gm., and presented a homogeneous purple appearance on section. The microscopic appearance was uniform in all instances. The malpighian bodies were small and widely separated. The venous sinuses were enlarged, widely dilated and frequently empty. The endothelium of the sinuses was prominent, the outline of the cells often bulging into the lumen. The pulp was a mass of closely packed red cells, and this diffuse infiltration was the most striking microscopic feature. There was neither increase in connective tissue nor visible increase in iron pigment. The so-called iron incrustations were frequently encountered, lying usually within the trabeculae. There was no visible evidence of phagocytosis of the red cells, and except for an occasional lymphocyte, one found nothing but red cells distending, distorting and dilating the pulp.

It is of interest in this connection that in a review of a large number of splenomegalies of various sorts, the only ones presenting this picture were in cases of this disease. It is also of importance that, of two spleens presenting this appearance, one was from a patient with the diagnosis of pernicious anemia, and the other from one with the diagnosis of Banti's disease. Subsequent clinical investigation revealed the fact that both patients had hemolytic jaundice of

the congenital type. In both instances, the diagnosis was made first by finding the characteristic features of the disease in the microscopic section of the spleen. In both instances, this diagnosis was confirmed by subsequent clinical investigation.

DISCUSSION

MAURICE N. RICHTER: Was any distinction made between the congenital and the acquired type of the disease?

WILLIAM P. THOMPSON: We have included under the so-called congenital type all the typical cases of hemolytic jaundice. Some of them apparently went back to childhood; some were associated with a familial incidence of the disease, and others were not. As far as we could see, the disease was typical in all the cases, whether congenital or acquired.

MAURICE N. RICHTER: Did you have any very young patients?

WILLIAM P. THOMPSON: The youngest was 9 years old.

UNUSUAL METASTASES OF MALIGNANT TUMORS. REPORT OF SIX CASES. ANGELO M. SALA and (by invitation) ELMA BARANY.

Two cases of carcinoma of the cervix are presented, with metastases in the lungs. One was in a 60 year old woman with a highly malignant anaplastic carcinoma; the other, in a 28 year old woman with a transitional cell carcinoma. Both patients were colored. The metastases in the lungs were found at autopsy.

The third case was that of a white man, 55 years old, with an epithelioma of the pharynx. Routine examination of sections of the left kidney post mortem disclosed an unsuspected metastasis from the pharyngeal growth.

The fourth case, also an epithelioma of the pharynx, was in a man 64 years old. Postmortem examination disclosed metastases to the liver and lungs.

The fifth was a case of recurrent epithelioma of the penis in a white man 36 years old. At autopsy there was found diffuse thickening of the intestinal serosa and of the splenic capsule. Grossly there was no suggestion of malignancy, but microscopically the picture was that of metastatic deposition (fig. 1).

The sixth case was an epithelioma of the tongue, with bilateral metastases to the cervical nodes, in a colored man 49 years old. At autopsy, extensive involvement of heart muscle was found. Two of the metastatic tumors were 3 cm. and 2 cm. in diameter, respectively. Smaller deposits were scattered throughout the myocardium, apparently following a coronary distribution (fig. 2).

These six cases, showing some of the decidedly less usual metastases, illustrate the necessity of painstaking search for evidences of metastasis in all cases of malignant growth. The first two cases offer an interesting contrast. In a 60 year old woman one does not usually see a rapidly growing anaplastic carcinoma of the cervix with distant metastases. In the second case, the cervical cancer was of the type usually encountered, from which one does not ordinarily expect distant metastases, except very late after onset. That both patients were colored may be of some significance. In the third case, renal metastasis from an epithelioma of the pharynx was neither expected nor suspected, and the discovery of it on our part must be regarded as a fortunate accident. Metastasis to the pulmonary parenchyma from an epithelioma of the pharynx, as presented in the fourth case, is certainly not common. The fifth case is remarkable because of the unusual dissemination of the metastases, manifesting themselves as a diffuse, not nodular, thickening of the intestinal serosa and splenic capsule. Only the microscope disclosed the true state of affairs in this case. Finally, metastasis to the heart from an epithelioma of the tongue is an extremely rare occurrence, and the case here reported is a brilliant example of the capricious behavior of malignant tumors with which we are at times confronted.

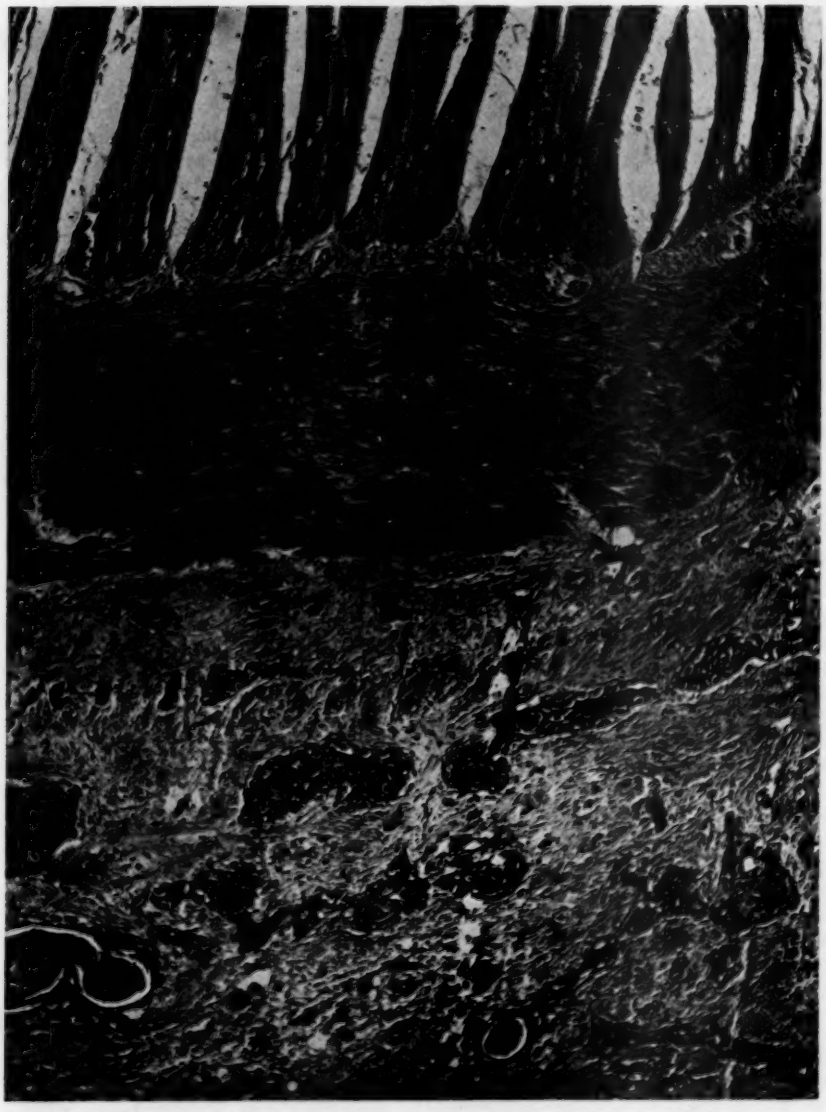

Fig. 1.—Intestinal serosa with metastatic squamous cell epithelioma; reduced from × 140.

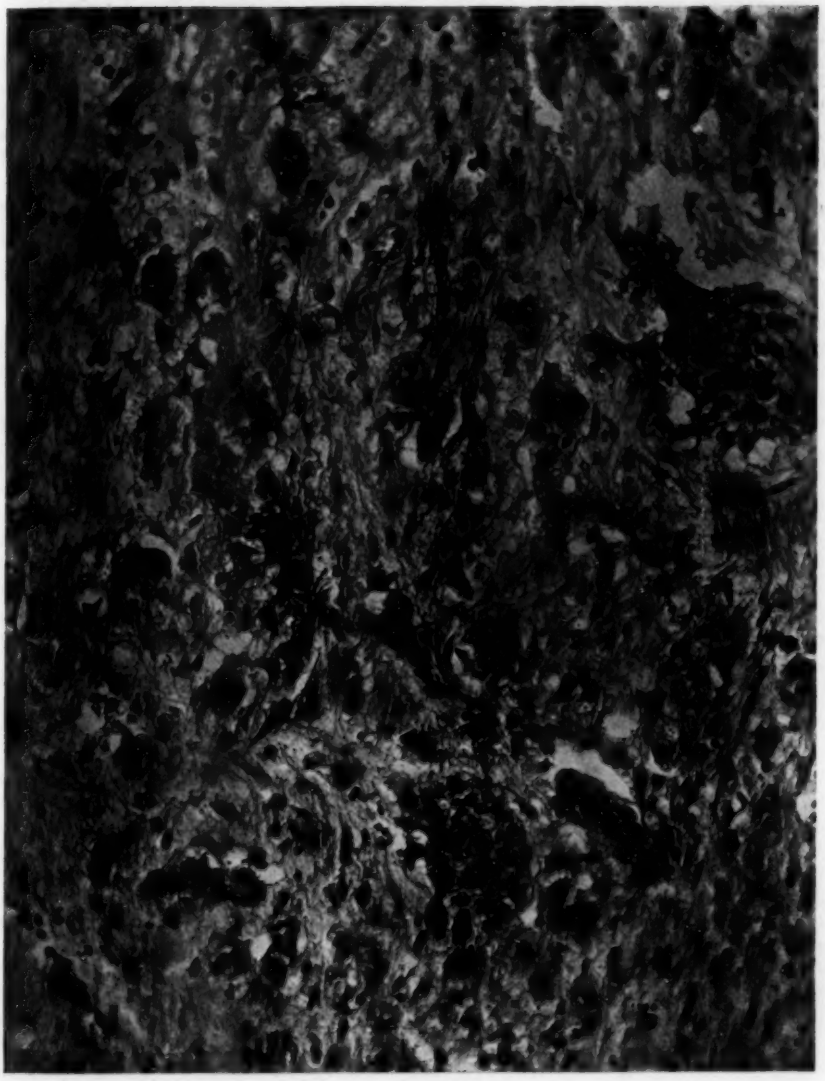

Fig. 2.—Metastatic squamous cell epithelioma in myocardium; reduced from × 280.

FACTORS INFLUENCING TYPES OF BONE RESORPTION. HENRY L. JAFFE and AARON BODANSKY.

Four litters of dogs, 3, 6, 8 and 18 months old, respectively, were given gradually increasing doses of ammonium chloride. Some of the litter mates in each age group received a diet in which calcium was high; others, a diet in which calcium was low. Some of those on each diet were given ammonium chloride, and some were retained as high and low calcium controls without ammonium chloride. The diet consisted of fresh lean horse meat supplemented in the case of the dogs receiving the diets in which calcium was to be high, with bone meal and calcium lactate (2.5 Gm. of each per kilogram of body weight), and in all cases with cod liver oil and tomato juice. Thus the calcium supplement amounted to from 0.5 to 2.5 Gm., depending on the size of the dog and on its food consumption. Once a week all dogs were fed cooked liver and kidney. The ammonium chloride, in 1 per cent solution, was given by stomach tube once a day at the beginning of the experiment; later, in order to avoid giving excessive volume at one time, the ammonium chloride was given twice a day. At the end of the experiment as much as 1 Gm. per kilogram was being given daily.

The animals were subjected to the experiment for eleven weeks. In the control dogs receiving the calcium supplement, the bones were normal in every respect. The low calcium controls showed thinning of the bones. The effects of the ammonium chloride treatment were observed in all the age groups, but were most striking in the younger animals. In a dog subjected to the experiment from the age of 3 months, a low calcium diet plus ammonium chloride led to fractures and deformities. Less striking changes were observed in a litter mate given ammonium chloride plus a high calcium diet.

In all age groups the gradation of changes was found to be strikingly dependent on the calcium intake. Those dogs receiving a diet in which calcium was high and ammonium chloride showed less osteoporosis than those receiving a diet in which calcium was low plus ammonium chloride or those receiving this diet without ammonium chloride. In the younger age groups a diet low in calcium to which ammonium chloride was added brought out more striking changes than a low calcium diet alone.

A CASE OF ACUTE MYELOID LEUKEMIA WITH UNUSUAL FEATURES. EDITH E. SPROUL (by invitation).

A 39 year old Englishwoman was admitted to the Presbyterian Hospital in a febrile condition, with a severe streptococcic lesion of the throat of one week's duration. The white count, at that time, totalled 2,500 cells per cubic centimeter, with the following differential count: polymorphonuclear leukocytes, 7 per cent; lymphocytes, 44 per cent, and unclassified cells, 47 per cent. The latter cells closely resembled the monocyte in nuclear configuration and inner pattern, but bore the cytoplasm of the myeloblast. Supravital staining revealed none of the rosettes of the monocyte. Peroxidase stains were positive.

The known course of the disease occupied twenty-seven days. There was a terminal rise in the white count to 89,000, with a differential count of myeloblasts of 47 per cent, and of myelocytes, 44 per cent; no eosinophils were noted. The spleen became enlarged and painful. Death followed rapid collapse, with an abrupt fall in the red cell count.

Postmortem examination established the immediate cause of death as hemorrhage into the peritoneal cavity following rupture of a large subcapsular hematoma of the spleen. There was bilateral lobular pneumonia with small abscess formation. Grayish foci of infiltration were visible grossly in the liver and kidneys. The lymph nodes were but slightly enlarged and soft. The thyroid gland was large. The bone marrow everywhere appeared dark red and moist.

Microscopically, the distribution of the visceral infiltration was not remarkable except for a predilection for the perivascular zones and an unusual intensity in

the portal areas of the liver, the uterus, suprarenal glands and thyroid gland. Eosinophilic myelocytes often formed more than 50 per cent of the infiltration, and were everywhere conspicuous, in the bone marrow, as well as in the viscera.

The case was regarded as unusual because of:

1. The appearance of atypical cells (monocytoid promyelocytes?) in the blood at the onset of the disease.

2. The presence of hematomas in the spleen with terminal rupture.

3. The discrepancy in character between the cells of the circulating blood and those infiltrating the tissues.

4. The preponderance of eosinophilic myelocytes in the tissues in what would otherwise appear to have been a case of acute myeloid leukemia.

DISCUSSION

NATHAN ROSENTHAL: This case of aleukemic leukemia or leukopenic leukemia is most unique. At first one would be inclined to classify it incorrectly as agranulocytic angina, and I was glad to hear that this was not done. Similiar cases have been classified as agranulocytic angina on account of the lack of a postmortem examination. Furthermore, the persistent presence of premature myeloid cells rules out such a condition. The question of whether they were monocytes or myeloblasts is not very important, although it is beginning to be recognized that there is a leukemia of the monocytic type. The predominating cells in monocytic leukemia show a negative oxidase reaction, indicating a premature type of monocyte. I do not know whether an oxidase stain was done on the monocytic cell found at first in the present case.

WILLIAM P. THOMPSON: These abnormal cells were oxidase-positive. There is one other interesting thing in connection with this case. The same day this patient came in, a young man was admitted with an identical clinical picture, a sore throat due to infection with a hemolytic streptococcus and the same blood picture. He got the better of his sore throat, and one week later the blood picture was normal, while that of our patient progressed to the terminal pathologic picture of leukemia. On admission these patients were identical clinically, presenting similar white counts and differential counts, including the presence of these cells that we do not know how to classify.

MAURICE N. RICHTER: I have had the opportunity of studying blood smears and sections in a number of cases diagnosed as monocytic leukemia, and I have been impressed by two things: one is that it is almost always possible to find transitional forms between the myeloblasts and the monocytes, and the other is that it is exceedingly difficult in sections to be sure that the monocytes are coming from reticulo-endothelial cells, as is generally reported. For these reasons I am a little skeptical as to the extent to which monocytic leukemia can be regarded as an entity. In regard to this particular case, I do not think now that the question of monocytic leukemia enters. This was only an abnormal feature of the blood. From the histologic standpoint it is purely a myeloid leukemia.

ANGELO M. SALA: We heard Dr. Sproul say that there was a streptococcus blood culture. Was any significance attached to that?

WILLIAM P. THOMPSON: No, just one flask showed a positive culture; the others did not. The throat cultures several times gave an almost pure growth of the hemolytic streptococcus.

NATHAN ROSENTHAL: With regard to monocytic leukemia, there have been more cases reported lately. I recently observed a case in which the blood cells were studied by the supravital stain, following the technic of Sabin. The typical rosette was present in most of the cells.

MAURICE RICHTER: What was the origin of the monocytes?

NATHAN ROSENTHAL: The cells are derived from the reticulo-endothelial system.

Book Reviews

Pathologie und Klinik in Einzeldarstellungen. Herausgegeben von L. Aschoff, H. Elias, H. Eppinger, C. Sternberg und K. F. Wenckebach. Band V: Mass und Zahl in der Pathologie. Von Professor Dr. Robert Roessle, Direktor des Pathologischen Instituts der Universität Berlin, und Dr. Frédéric Roulet, Oberarzt am Pathologischen Institut der Universität Berlin. Paper. Price, 16 marks. Pp. 144, with 27 illustrations. Berlin: Julius Springer, 1932.

This book consists of a series of monographs, of which the following have been published: "Der appendicitische Anfall" by Aschoff; "Gewebsproliferation und Säurebasengleichgewicht" by R. Bálint and S. Weiss of Budapest; "Die Lebensvorgänge im normalen Knorpel und seine Wucherung bei Akromegalie" by J. Erdheim; "Thrombose" by A. Dietrich of Tübingen, and now "Mass und Zahl" by Roessle and Roulet. Roessle said that he was pleased to undertake the task proposed to him, of contributing to the series, because it naturally brought about an assay of the measurements he had made at postmortem examinations during many years, and he included with his own those made by William Müller, his predecessor at Jena. The opportunity was presented of determining the value of such estimates, of passing on to others information useful to them in their own work and of filling a real gap in anthropometry of human viscera.

The suggestion for a monograph on "body weight, organ weight and the caliber of vessels" undoubtedly had its birth in the numerous investigations completed by Roessle and his assistants during the past decade or two: studies of the growth and age of large arteries and their relation to disease of the blood vessels; the pathology of body growth; the pathology of growth in children; growth of the internal organs in children; characteristics of mortality in Basel, a contribution to geographic pathology, and his "Technic der Obduction mit Einschluss der Mass-method" in the "Abderhalden Handbuch." Pathologists are not in accord as to the importance of accurate measurements as a routine in postmortem examinations, although their desirability in medicolegal inquiries or when the bodies are immature is generally admitted. Roessle believes that they are an aid to scientific diagnosis and play a disciplinary rôle, and that they are useful to check the accuracy of anatomic diagnoses and to detect inconsistencies in protocols. In discussing methods, he reviews many sources of error, such as the escape of blood from the liver and lungs and the mistake of including hilar tissues, subepicardial fat and clots of blood in the heart with organ weights. Terminal edema of the lungs and fluids in them from gravity have so interfered, that it is doubtful whether the weight of normal lungs is known. Some of these details and others bearing on the same topic are mentioned in his account of postmortem technic.

This volume is a profusion of statistics in tables like those in that indispensable and time-honored thesaurus, Vierordt's "Daten und Tabellen"; indeed, as an expansion of the familiar standards of the older work, those assembled by Roessle and Roulet possess an importance of the first order. In Vierordt's work, it will be recalled, there is no discussion of the values displayed in tables. In "Mass und Zahl," however, tables are an appendage to text, and personal observations are included and contrasted with determinations made by other investigators and reported, for the most part, since the last edition of "Daten und Tabellen" in 1906. The entire range, prenatal and postnatal, of human life is covered. Although there are no tables devoted solely to weights of organs in various types of disease, a few are wholly concerned with "organ correlation." The weight of the heart is compared with body length, body weight and the weight of some viscera and of a biceps muscle. In another table, weights of the liver, spleen, pancreas and body are correlated; in a third, those of the testes and suprarenal glands.

Physical anthropology has been overwhemmingly occupied with the exterior of the body and with the skeleton so far as it contributes to shape. Knowledge of the viscera of the separate human races is in its infancy. The information Roessle has contributed was from his work at Jena in middle Germany, with the agrarian people or workmen of the better class, and from that at Basel, with its proletarian and cosmopolitan inhabitants; other estimates were a fruit of the World War. Investigations by Americans are included in the several concluding pages of bibliography: Scammon, Mall, Wilder, Moon, A. W. Meyer, Benedict and Talbot. Measurements by some of them are included in the tables. The usual list of contents follows the preface, but unfortunately there is no index. The last table contains weights in bodies selected because the form of death promised normal weights: eighty-seven bodies of males and twenty-eight of females, with practically all ages represented. Evidently a prolonged overhauling of records was required to make the selection, because the period during which the bodies were examined covered thirteen years. One of the interesting conclusions is that in no single body is there what might be termed an ideal average for all the computations, weights of important organs, body weight and length, trunk circumferences and the like. The authors hope that perhaps the data offered may stimulate others to furnish measurements so that ultimately indexes and ratios shall be available pointing definitely to types of constitution or of disposition to particular forms of disease. They look forward to a time when records shall be so abundant that "gland formulas" may be employed to explain why in some bodies of persons killed outright wide variations from average weights, volume and the like are present, notwithstanding that there is no evidence of disease, and they suggest that what they have presented is a step in that direction.

Books Received

RECENT ADVANCES IN PATHOLOGY. By Geoffrey Hadfield, M.D., F.R.C.P. (Lond.), Professor of Pathology in the University of London, Pathologist to the Royal Free Hospital, and Lawrence P. Garrod, M.A., M.B., B.Ch. (Camb.), M.R.C.P. (Lond.), Bacteriologist and Lecturer in Bacteriology, Late Demonstrator of Pathology, St. Bartholomew's Hospital. Price, $3.50. Pp. 392, with 67 illustrations. Philadelphia: P. Blakiston's Son & Co., 1932.

REPORTS OF THE COMMITTEE UPON THE PHYSIOLOGY OF HEARING: I. STUDIES IN THE LOCALIZATION OF SOUND. A. THE LOCALIZATION OF SOUNDS IN THE MEDIAN PLANE. By J. H. Shaxby and F. H. Gage. B. SOME FACTORS IN AUDITORY LOCALIZATION. By H. E. O. James and Marion E. Massey. Medical Research Council, Special Report Series No. 166. Price, 1 shilling, net. Pp. 51. London: His Majesty's Stationery Office, 1932.

ALCOHOL AND INHERITANCE: AN EXPERIMENTAL STUDY. By F. M. Durham and H. M. Woods, Medical Research Council, Special Report Series No. 168. Price, 1 shilling, 3 pence, net. London: His Majesty's Stationery Office, 1932.

IMMUNITÄT, ALLERGIE UND INFEKTIONSKRANKHEITEN: PRAKTISCHE ERGEBNISSE DER WISSENSCHAFTLICHEN FORSCHUNG UND KLINISCHEN ERFAHRUNG. Herausgegeben von Rudolf Degkwitz, Erich Leschke, Hans Schlossberger, Georg Schröder und W. Storm van Leeuwen. Schriftleitung: E. Michelsson. Band III, Heft 4-6. Sonderheft. Grundfragen der Immunbiologie und Allergielehre. Price, paper, 7.50 marks. Pp. 133-253. Munich: Verlag der Ärztlichen Rundschau Otto Gmelin, 1932.